AFFIDAVIT

I, Dr. William Henry Kerr Anderson, a citizen of the United Kingdom, residing at 5112 Cortland Street, Midland, MI 48642, declare and state as follows:

1. I have received a Ph.D. in Biochemistry from the University of Aberdeen, United Kingdom. I have worked for the Dow Chemical Company ("TDCC") for over 20 years, during which I have been involved in biotechnology. I currently have the title of Technical Director & Scientist of Plant Biopharmaceuticals for TDCC. In that capacity, I oversee all of TDCC's research and development in the area of plant-made pharmaceuticals. A copy of my curriculum vitae is attached.

2. **WO 00/34490.** I have read and am familiar with the specification, figures and claims of international patent application number PCT/JP99/06881, published as international publication number WO 00/34490. TDCC has acquired a global, exclusive assignment to the invention disclosed and claimed in WO 00/34490.

The WO 00/34490 patent application discloses and claims novel methods of using plant cells to produce (*i.e.*, manufacture) glycoproteins having a human-type sugar chain, as well as the resultant transformed plant cells, transformed whole plants and glycoproteins produced from such methods. In one embodiment, the patent application discloses and claims a method in which a transformed plant cell is obtained by introducing into a plant cell the gene of a glycosyltransferase and the gene of an exogenous glycoprotein, which transformed plant cell is then cultivated. The efficacy of the disclosed materials and methods is demonstrated by Example 1 through Example 7 of WO 00/34490, which establish, *inter alia,* the successful placement of galactose on N-linked glycan structures of horseradish peroxidase in agrobacterium-transformed tobacco cells expressing human beta-1,4-galactosyltransferase ("GalT") (*i.e.*, a glycosyltransferase) and horseradish peroxidase (*i.e.,* an exogenous glycoprotein), both of which expressions were driven by the cauliflower mosaic virus 35S ("CaMV 35S") promoter.

To confirm and further demonstrate the efficacy of the novel materials and methods disclosed and claimed in WO 00/34490, additional experiments that are fully supported by the disclosure of WO 00/34490 were carried out under my direction and control. These additional experiments utilize materials and methods that were publicly known before the December 9, 1998 priority date of the application. By way of an overview, these additional experiments establish the successful placement of galactose on N-linked glycan structures of human chorionic gonadotropin ("HCG") in Whiskers®-transformed corn cells and regenerated transformed corn plants expressing human GalT and human HCG, both of which expressions were driven by the maize ubiquitin 1 promoter. Thus, these additional experiments further support the claims in WO 00/34490 for methods of using transformed plant cells and transformed whole plants to produce (*i.e.*, manufacture) a glycoprotein having a human-type sugar chain.

3. **Experimental Overview.** As detailed below and in the attached Exhibits, two vector constructs each including a gene for the β-chain of human chorionic gonadotropin (HCG∃) (*i.e.*, an exogenous glycoprotein) were introduced into corn (maize) (*Zea mays* L.) callus tissue, which were regenerated into whole plants. The first construct (designated "pDAS920") expresses only the gene for HCGβ. The plant cells and whole plants transformed with pDAS920 are designated as "HCGβ-only" below and acted as the control in these experiments. The second construct (designated "pDAS940") co-expresses the HCGβ gene with a gene for human β1,4-galactosyltransferase ("GalT") (*i.e.*, a glycosyltransferase). The plant cells and whole plants transformed with pDAS940 are designated as "HCGβ-GalT" below.

Support for the materials used in these experiments can be found throughout WO 00/34490 including as follows:

> "maize" (corn) is disclosed as one of the "preferred" or "ideal" plants to use in the invention at page 14, lines 22-27, and specifically at page 14, line 24;
>
> "human choriogonadotropin (HCG)" is disclosed as an example of one of the "exogenous glycoproteins" to use in the invention at page 15, line 22 to page 16, line 13, and specifically at page 16, line 1; and

"galactosyltransferase" is disclosed as a "specific example" of "an enzyme capable of conducting a transfer reaction of a galactose residue to a non-reducing terminal acetylglucosamine residue" at page 14, line 29 to page 15, line 7, and specifically at page 15, line 4. Even more specifically, "human β1,4 Galactose Transferase" or "GalT" is disclosed at page 18, lines 13-17 and throughout Examples 1-7.

The expression of galactosylated N-glycan chemical structures of HCGβ was detected in the HCGβ-GalT-transformed corn callus and plants, in contrast to the results obtained with the HCGβ-only transformants where no such expression was found. Thus, the experiments discussed in this affidavit provide additional support for methods of using plant cells to produce (*i.e.*, manufacture) glycoproteins having a human-type sugar chain as disclosed and claimed in WO 00/34490.

4. **Experimental Protocol.** This section describes the materials and methods of the additional experiments that provide further support for the invention disclosed and claimed in WO 00/344990. All of the materials and methods described below are based on the disclosure of WO 00/34490 and information that was publicly available before December 8, 1998, the earliest priority filing date of WO 00/34490.

Production of Vector Constructs. Vector pDAS940 (Exhibit A) comprised GalT;[1] HCGβ;[2] and the selectable marker was a synthetic, plant codon usage optimized phosphinothricin acetyltransferase coding sequence ("PAT"), originally from *Streptomyces viridochromogenes*.[3] Vector pDAS920 (Exhibit B) comprised the same coding sequences for HCGβ and PAT, but did not contain the coding sequence for GalT. The expression of GalT and HCGβ in both vectors

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[1] Genbank Accession No. X14085, submitted April 13, 1995.

[2] Genbank Accession No. J00117, submitted April 10, 1996.

[3] Genbank Accession No. A29201, submitted in 1995; White et al. "A Cassette Containing the BAR gene of *Streptomyces hygroscopicus*: A Selectable Marker for Plant Transformation," *Nucleic Acids Research*, Vol. 18, No. 4, p. 1062 (1990); Wohlleben et al., "Nucleotide Sequence of the Phosphinothricin N-acetyltransferase Gene from *Streptomyces viridochromogenes* Tue494 and its Expression in *Nicotiana tabacum*," *Gene*, Vol. 70, pp. 25-37 (1988).

was driven by the maize ubiquitin 1 promoter.[4] PAT expression was controlled by rice actin promoter (+ intron).[5] Other vector components included *Zea mays* ("Zm") per5 3' UTR[6]; maize lipase 3' UTR[7]; and RB7 MARs.[8] The vector backbone was derived from modified pUC19.[9]

The DNA sequences of both the GalT and HCGβ genes were codon biased and redesigned for better expression in plants. The codon bias of these two genes was based on the codon usage

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[4] Christiansen *et al.*, "Maize Polyubiquitin Genes: Structure, Thermal Perturbation of Expression and Transcript Splicing, and Promoter Activity Following Transfer to Protoplast by Electroporation", *Plant Molecular Biology*, No. 23, pp. 675-689 (1992); Schledzewski *et al.*, "Quantitative Transient Gene Expression: Comparison of the Promoters for Maize Polyubiquitin 1, Rice Actin 1, Maize-Derived Emu and CaMV 35s in Cells of Barley, Maize and Tobacco", *Transgenic Research,* No. 3, pp. 249-255 (1994).

[5] McElroy *et al.*, "Isolation of an Efficient Actin Promoter for Use in Rice Transformation", *Plant Cell*, No. 2, pp.163-71 (February 1990); McElroy, *et al.*, "Construction of Expression Vectors Based on the Rice Actin 1 (Act1) 5' Region for Use in Monocot Transformation", *Molecular and General Genetics,* No. 1, pp. 150-60 (Dec. 1991).

[6] Folkerts *et al.*, "Identification, Isolation, and Molecular Characterization of cDNA Clones and Genomic Clones Encoding a Root-Preferential Peroxidase of Maize (*Zea mays L.*)", Biotechnology Dept., Dow AgroSciences, U.S.A., Indianapolis, IN, DERBI 43910 (1993); Armstrong et al. "Transient Analysis of a Root Specific Promoter from a Maize Peroxidase Gene", Biotechnology Department, Dow AgroSciences U.S.A., Indianapolis, IN, DERBI 45413 (1995); and Armstrong et al., "Discovery and Testing of Propriety 3' Untranslated Regions from Maize for Expression of Foreign Genes", Biotechnology Department, Dow AgroSciences U.S.A., Indianapolis, IN, DERBI 64422 (June 1998).

[7] Armstrong et al., "Discovery and Testing of Propriety 3' Untranslated Regions from Maize for Expression of Foreign Genes", Biotechnology Department, Dow AgroSciences U.S.A., Indianapolis, IN, DERBI 64422 (June 1998); Paek *et al.*,"Inhibition of germination gene expression by Viviparous-1 and ABA during maize kernel development", Mol. Cells 8(3), 336-342 (1998).

[8] Thompson et al., "A plant nuclear scaffold attachment region which increases gene expression", PCT Int. Appl. WO 9727207 (1997); Ulker, *et al.*, "A tobacco matrix attachment region reduces the loss of transgene expression in the progeny of transgenic tobacco plants", Plant J. 18(3), 253-263 (1999).

[9] Yanisch-Perron *et al.*, "Improved M13 phage cloning vectors and host strains: nucleotide sequences of the M13mp18 and pUC19 vectors", Gene 33 (1), 103-119 (1985); Chambers *et al.*,"The pMTL nic- cloning vectors. I. Improved pUC polylinker regions to facilitate the use of sonicated DNA for nucleotide sequencing", Gene 68 (1), 139-149 (1988); Norrander *et al.*, Gene 26: 101-106 (1983).

called "hemicot", which represents an intermediate compromise between maize and dicot plants. The following modifications were made while the amino acid sequence remained unchanged:

Removed mRNA destabilizing elements and other sequence patterns as listed in Exhibit C.
Removed restriction enzyme sites to facilitate cloning and molecular analysis.
Retained A/T runs of at least 6 base pairs in the native sequence.
Removed alternative open reading frames that are at least 250 bp.
Optimized codons based on hemicot codon usage.
Made GC% uniform throughout the gene.
Reduced the di-nucleotide frequency of "CG" and "TA"
Increased the di-nucleotide frequency of "TG" and "CT".
Removed stem-loop structures with free energy of at least -12.

Each of the above steps were conducted repetitively until all aspects reached their optimal state.

Gene synthesis was carried out at Picoscript™ (HCGβ) and Retrogene™ (GalT), respectively.

Vector 1 (pDAS920) (Exhibit B), containing the HCGβ gene without the GalT gene, was constructed as follows:

The HCGβ gene was recovered from pCR4.0 TOPO plasmid supplied by Picoscript™ as a Nco1-Sac1 fragment and moved into plasmid pDAB4005 (ZmUbi1 /GUS /ZmPer5 3'UTR) which had been cut with Nco1 and Sac1, so that the HCGβ gene replaced GUS to form plasmid pDAB 8536, forming the HCGβ expression cassette (ZmUbi1/HCGβ/ZmPer5 3'UTR). The HCGβ expression cassette from pDAB8536 was recovered as a Not1 fragment and inserted into the Not1 site of the MAR/selectable marker plasmid pDAB 8504 to form Vector 1 (pDAS920). (Note: In Exhibit B, HCGβ is designated as "hCGb hv5 (final)".)

Vector 2 (pDAS940) (Exhibit A), containing both the HCGβ and GalT genes was constructed as follows:

The GalT gene was recovered from PCR-Blunt plasmid supplied by Retrogene™ as a BbsI - Sac1 fragment, and moved into plasmid pDAB4005 (ZmUbi1 /GUS /ZmPer5 3'UTR) which had been cut with Nco1 and Sac1, so that the GalT gene replaced GUS to form plasmid pDAB 8535, forming the GalT expression cassette (ZmUbi1/HCGβ/ZmPer5 3'UTR). The GalT expression cassette from pDAB 8535 was recovered as a Not1 fragment, blunt-ended by fill-in with T4 DNA Polymerase, and inserted into the Srf1 site of the Mar/selectable marker base vector (pDAB8504, to create pDAB8538). The HCGβ cassette was isolated from pDAB 8536 as a Not1 fragment, and

inserted into the Not 1 site of pDAB 8538 to create the final vector pDAS940. (Note: In Exhibit A, HCGβ is designated as "hCGb hv5 (final)" and GalT as "GalT F&S".)

DNA Preparation for Cell Transformation. *E. coli* strain DH5α carrying plasmid pDAS920 or pDAS940 was grown up in 2 liters of LB medium with ampicillin selection and cells were pelleted by centrifugation. Plasmids were purified with Qiagen's Plasmid Giga Kit to produce 10 mg of each plasmid.

Maize Cell Transformation and Selection. Each purified DNA plasmid preparation of pDAS920 and pDAS940 was introduced into *Zea mays* embryogenic suspension cultures by Whiskers® transformation, essentially as described in publications.[10] Whiskers®-treated cells were from regenerable suspension cultures derived from crosses of A188 X B73.[11]

Briefly, cells were collected from GN6 medium (N6 medium with 100 mg/L myo-inositol, 2mg/L 2,4-D, and 30 g/L sucrose), pretreated with osmotically enhanced medium GN6 SM (GN6 plus 0.25 M sorbitol and 0.25 M mannitol) for 30 minutes, mixed with DNA and sterilized silicon carbide fibers (Silar® SC-9, Advanced Composite Materials Corp., Greer, SC) in a prescribed ratio (36 ml cells packed cell volume + 50ml GN6 SM medium + 170 μg plasmid DNA + 100 ul Whiskers® solution, 5% w/vol), and vigorously agitated for 10 seconds. Cells were allowed to "rest" for a recovery period of two hours on the shaker in the same medium that had been diluted by a half volume of GN6 to reduce the osmoticum. Cells were collected on a sterile filter and cultured without selection on solid GN6 medium (containing 2 g/L Gelrite) for 1 week. The filter was then moved to GN6(1H) selective solid medium, containing 1 mg/L of the selective agent bialaphos, supplied from the formulated herbicide Herbiace® (Meiji Seika, Tokyo, Japan). Two weeks after transformation, cells were scraped off the filter and embedded in GN6 (1H) medium with 7% SeaPlaque® agarose, layered over solidified GN6(1H).

[10] Frame *et al.*,. "Production of fertile transgenic maize plants by silicon carbide whisker-mediated transformation", *Plant J.* 6(6): 941-948 (1994); Thompson *et al.* "Maize transformation utilizing silicon carbide whiskers: a review", Euphytica 85:75-80 (1995).

[11] Armstrong *et al.*, "Development and availability of germplasm with high Type II culture response", *Maize Genet. Coop. News Lett.* 65:92-93 (1991).

Bialaphos-resistant callus isolates were identified by growth in this medium and picked off 7-9 weeks after transformation. Individual isolates were numbered and subcultured every two weeks on solid GN6 (1H). These callus isolates were used in the subsequent molecular and biochemical experiments.

5. **Molecular Analysis of Callus Isolates.** Samples of the bialaphos-resistant callus isolates were used to prepare genomic DNA using Qiagen's DNeasy® 96 Plant Kit and the isolated genomic DNA was analyzed for the presence of the transferred genes in each individual callus isolate.

A PCR method was used to assess the presence of the HCGβ gene in isolates transformed with Vector 1 (pDAS920), and similarly to examine the presence of the HCGβ gene and the GalT gene in isolates made with Vector 2 (pDAS940).

The following primers were used to analyze the presence of HCGβ gene:

Forward: CTGTCCCGTTTGCATAACC
Reverse: AGGAGTCTTGGAATCTGGGA
Expected size: 290 bp
PCR conditions: denature at 94° C for 3 min followed by 35 cycles of 94° C 30 seconds, 60° C 30 seconds and 72° C for 30 seconds. The amplification reaction was completed with 10 min extension at 72° C.

For the analysis of GalT, the following primers were used:

Forward: TCCCACAACTTGTCGGTGT
Reverse: CAATCCTGTCGAACCTCTGAG
Expected size: 903 bp
PCR conditions: denature at 94° C for 3 min followed by 35 cycles of 94° C 30 seconds, 60° C 30 seconds and 72° C for 1 min. The amplification reaction was completed with 10 min extension at 72° C.

6. **Identification of HCGβ Expressing Maize Cell Colonies.** Callus samples from individually isolated unique transgenic events were extracted as follows. Samples from each event were fresh frozen in 96-well cluster tube boxes (Costar® 1.2 ml polypropylene, with lid) along with a steel and a tungsten bead in each well. 450 μl of extraction buffer (PBS, Sigma® #

P3813 and 0.05% Tween 20™) was added per well and the box of samples was pulverized for 4 minutes full speed on a Kleco Bead Mill. The plate was centrifuged (4° C -15° C) at 2500 rpm for 10 minutes. Extracts were removed to a 96-well deep well plate and frozen for storage. All screening assays were performed on these extracts of individual events.

ELISA assays for presence of free HCGβ were performed according to the manufacturer's instructions using a kit (Product # P 2016) from American Research Products, Inc., Belmont, MA.

7. **Purification of Transgenically Expressed HCGβ From Corn Cells.** The following reagents were purchased from Sigma®: recombinant HCGβ purified from a mouse cell line (Catalog # C-6572); monoclonal anti-HCGβ from mouse ascites fluid, clone PC-2 (Catalog # C-7659); polyclonal goat anti-mouse IgG alkaline phosphatase conjugate (Catalog # A-3562); polyclonal rabbit anti-goat IgG horse radish peroxidase conjugate (Catalog # A5420); protease inhibitor cocktail for plant cell extracts (Catalog # P 9599). The following antibodies were purchased from BioDesign International: monoclonal anti-HCGβ antibody (Catalog # E20106M) and polyclonal goat anti-HCGβ antibody (Catalog # D82901G). An ELISA kit for screening and measuring free HCGβ (Catalog # P-2016) was purchased from American Research Products, Inc.™ Acti-gel Superflow resin was purchased from Sterogene Bioseparation Inc.

Callus isolates having all of the following characteristics were pooled and fresh-frozen on dry ice for further testing: 1) they had been transformed with HCGβ vector pDAS920; 2) they had positive PCR detection of the HCGβ gene; and 3) they were expressing HCGβ by ELISA. The selected calli were thoroughly mixed during and after freezing. The pooled sample was stored at -80° C until used for protein isolation.

Frozen corn calli were removed from -80°C and weighed in a plastic bag and 200 ml of freshly prepared extraction buffer (0.65 M NaCl, 3 mM KCl, 5 mM EDTA, 0.1% Triton X-100, pH 7.5) was added to 60 g (wet weight) of plant tissue. Next, 2 ml of Sigma® protease inhibitor cocktail solution was added to the mixture. The suspension was transferred into a Bead-Beater device

(BioSpec Products, Inc., Bartlesville, OK) containing 200 ml 1:1 ratio mixture of 1 mm Zirconia/Silica beads and 0.1 mm Glass beads, and homogenized on ice for 1 min each round, with 1 min interval break, for total of 7 rounds. The supernatant of extract was poured into a 250 ml centrifuge bottle, and then centrifuged for 25 min at 23,420 x g to pellet insoluble material. The supernatant was sequentially filtered through fiber glass paper, 0.45 μm and 0.2 μm filter unit, and applied to affinity purification at same day of preparation.

Approximately 30 mg of anti-HCGβ subunit monoclonal antibody (BioDesign catalog # E20106M, clone ME.106) was coupled to 5 ml of Acti-gel Superflow resin according to the manufacturer's instructions, and the matrix was packed into an Amersham Biotech HR16/5 column. Another 5 ml Acti-gel Superflow resin was chemically blocked (but without antibody) and was put into a tandem column to serve as a pre-column before the affinity column during the affinity purification step.

Corn callus protein extract (approximately 800 ml each for the transformation event pool from construct pDAS920, with total two batches prepared) were passed and recirculated overnight through the 10 ml tandem columns described above with a peristaltic pump at a flow-rate of 1.5 ml/min. The columns were washed sequentially with 100 ml of loading buffer (same as the extraction buffer) and 100 ml of PBS (0.15 M NaCl, 3 mM KCl). The pre-column was then removed, and the bound protein was eluted from the affinity column with approximately 2.5 column volumes of 0.1 M Sodium Citrate/0.3 M NaCl, pH 3.0 at a flow-rate of 0.5 ml/min, and immediately neutralized with 1/10 volume of 1 M Tris-HCl. pH 9.0. Then, 1 ml fractions that were eluted were collected, and monitored by 280 nm using an AKTA Explorer. Peak fractions were also evaluated by SDS-PAGE and confirmed by ELISA measurement. The pooled elutes were concentrated to a small volume by using Millipore 5 kDa MWCO centrifugation filter unit.

By this method, approximately 2,200 μg HCGβ from the pDAS920 calli pool and 160 μg HCGβ from the calli pool transformed with pDAS940 were obtained, as estimated by a micro BCA assay using BSA as a standard (BCA = bicinchoninic acid; BSA = Bovine serum albumin).

N-terminal sequencing (Applied Biosystems Procise HT, Edman chemistry) on the eluted HCGβ from the pDAS920 calli pool confirmed 30 amino acid residues of the expected HCGβ sequence. Peptide mass fingerprinting identified fragments corresponding to a total of 75 amino acid residues of the expected HCGβ sequence, which represents over 50% of the mature processed HCGβ protein. Some proteolytic degradation products of HCGβ were also recovered. The elution profiles of HCGβ from the pDAS920 and pDAS940 preparations were comparable, as were the silver stained SDS-PAGE gels. Finally, the identity of the eluted protein was demonstrated as HCGβ based on affinity purification by one anti-HCGβ monoclonal antibody, ELISA quantitation by a second anti-HCGβ antibody, and staining by a third anti-HCGβ antibody in Western analyses.

8. Northern Analysis of pDAS940 Callus Samples (HCGβ-GalT). Thirty seven individual events which arose from transformation with pDAS940, and which had been rated positive for HCGβ expression, were analyzed for expression of the GalT gene at the RNA level by Northern analysis of callus tissue.

A conventional Northern blot analysis procedure was followed. Briefly, total RNA was extracted from callus samples using RiboPure™ kit (Ambion, Inc). Next, 10 μg of total RNA from each event and a non-transgenic callus sample as well as an RNA size marker were loaded onto a formaldehyde denatured gel and subjected to electrophoresis. The gel separated RNA was transferred onto a nylon membrane overnight and RNA samples were fixed to membrane through UV cross-linking. The membrane was then prehybridized in UltraHyb solution (Ambion, Inc) for 30 min and hybridized with a ^{32}P-labeled GalT DNA probe for overnight. The unhybridized probe was washed away in a series of wash buffers with gradually increased stringency, from 2xSSC at 42° C to 0.1xSSC at 65° C. An X-ray film was exposed at -80° C for 2 hours.

As shown in the table immediately below, 19 of 37 events (51%) had a GalT transcript with the expected size. Fifteen of the 19 events had very strong expression as indicated by strong bands, while the other four events had relatively low expression of GalT. Two other events had

transcripts with two alternative sizes, one smaller and the other larger than expected size; one other event had a truncated transcript with a band smaller than the expected size; and 15 other events had no GalT transcript detected.

Summary of Northern blot analysis of pDAS940 transgenic callus.

No. of total events	No. events with expected size	No. of events with larger bands	No. of events with smaller bands
37	19 (15 strong + 4 weak)	3	2

9. Preparation of Callus Pool from Northern-Positive pDAS940 Callus. A new pool of tissue containing the 15 GalT Northern-positive events was prepared by combining and freezing the callus as described above.

10. Plant Regeneration from Transgenic Maize Callus and Plant Scoring for HCGβ Expression. Regeneration was initiated by transferring callus tissue to culture dishes containing a cytokinin-based induction medium, which contains Murashige and Skoog salts and vitamins,[12] 30 g/L sucrose, 100 mg/L *myo*-inositol, 5 mg/L 6-benzylaminopurine (BAP); 0.025 mg/L 2,4-D; 1 mg/L bialaphos or Herbiace®; and 2.5 g/L Gelrite® at pH 5.7. The cultures were placed in low light (125 ft-candles) for one week followed by one week in high light (325 ft-candles). Following this two-week induction period, tissue was transferred to hormone-free regeneration medium (which was identical to the induction medium except that it lacks 2,4-D and BAP) and kept in high light. Small (1.5 cm to 3 cm) plantlets were removed and placed in 150x25 mm culture tubes containing SH medium (SH salts and vitamins,[13] 10 g/L sucrose, 100 mg/L myo-inositol, 5 mL/L FeEDTA, and 2.5 g/L Gelrite®, pH 5.8). Plantlets were transferred to 10 cm pots containing approximately 0.1 kg of Metro-Mix 360® (The Scotts Co. Marysville, OH) in the greenhouse as soon as they exhibited growth and developed a sufficient root system. They were grown with a 16 hour photoperiod supplemented by a combination of high pressure sodium

[12] Murashige, T. and F. Skoog, Physiol. Plant 15: 473-497 (1962).

[13] Schenk, R.V. and A.C. Hildebrandt, "Medium and techniques for induction and growth of monocotyledonous and dicotyledonous plant cell cultures", Can. J. Bot. 50:199-204 (1972).

and metal halide lamps, and watered as needed with a combination of 3 independent Peters Excel® fertilizer formulations (Grace-Sierra Horticultural Products Company, Milpitas, CA). At the 3-5 leaf stage, plants were transferred to 5 gallon pots.

Leaf samples from individual plants (regenerated from HCGβ-expressing callus events) were extracted as follows. Samples from each plant were fresh frozen in 96-well cluster tube boxes (Costar® 1.2 ml polypropylene, with lid) along with a steel and a tungsten bead in each well. 450 μl of extraction buffer (PBS, Sigma # P3813 and 0.05% Tween 20®) was added per well and the box of samples was pulverized for 4 minutes full speed on a Kleco Bead Mill®. The plate was centrifuged (4° C -15° C) at 2500 rpm for 10 minutes. Extracts were removed to a 96-well deep well plate and frozen for storage. All screening assays were performed on these extracts of individual events.

ELISA assays for presence of free HCGβ were performed according to the manufacturer's instructions using a kit (Product # P 2016) from American Research Products, Inc., Belmont, MA.

11. <u>Affinity Column Purification of HCGβ from Callus of pDAS940 Northern Positives.</u>
Thirty five grams of calli were obtained from the pool of 15 callus events which tested positive for HCGβ expression and GalT-RNA expression.

Frozen corn calli were removed from -80°C and weighed in a plastic bag. Next, 200 ml of freshly prepared extraction buffer (0.65 M NaCl, 3 mM KCl, 5 mM EDTA, 0.1% Triton X-100, pH 7.5) was added to 35 g (wet weight) of plant tissue, and 2 ml of Sigma® protease inhibitor cocktail solution was then added to the mixture. The suspension was transferred into a Bead-Beater device (BioSpec Products, Inc., Bartlesville, OK) containing 200 ml of 1 mm Zirconia beads, and homogenized on ice for 1 min each round, with 1 min interval break, for total of 7 rounds. The supernatant of extract was poured into a 250 ml centrifuge bottle, and then centrifuged for 25 min at 23,420 x g to pellet insoluble material. The supernatant was

sequentially filtered through fiber glass paper (0.45 μm and 0.2 μm filter unit) and affinity purified on the day of preparation.

The clarified extract was applied to the affinity column (described above) which had been washed with 20 ml freshly prepared elution buffer and then equilibrated with extraction buffer (same as binding buffer).

Corn callus protein extract was passed and recirculated overnight through the 10 ml tandem columns described above with a peristaltic pump at a flow-rate of 1.5 ml/min. The columns were washed sequentially with 100 ml of loading buffer (same as the extraction buffer) and 100ml of PBS (0.15 M NaCl, 3 mM KCl). The pre-column was then removed, the bound protein was eluted from the affinity column with approximately 2.5 column volumes of 0.1 M Sodium Citrate/0.3 M NaCl, pH 3.0 at a flow-rate of 0.5 ml/min, and immediately neutralized with 1/10 volume of 1 M Tris-HCl. pH 9.0. One milliliter fractions eluted were collected, and monitored by 280 nm using an AKTA Explorer. Peak fractions were also evaluated by SDS-PAGE and confirmed by ELISA measurement. The pooled elutes were concentrated to a small volume by using Millipore 5 kDa MWCO centrifugation filter unit.

Elution profiles and silver stained SDS-PAGE results were consistent with prior HCGβ isolations from callus tissue. ELISA quantitation indicated that 135 μg HCGβ were obtained from the Northern-positive pool of pDAS940 calli.

12. **<u>Purification of Plant-Expressed HCGβ With and Without GalT.</u>** Young plants were collected from the greenhouse representing events which had been transformed with pDAS920, and those which had tested positive for expression of HCGβ in callus and again in the plants regenerated from the callus were selected.

A 100 gm sample of frozen young plants (without roots) was broken up and divided into two parts of about 50 grams each. To each sample was added 200 ml of extraction buffer. After pulverizing with 1 mm Zirconia beads as before, the extract was filtered through a glass membrane, centrifuged at 12,000 rpm for 20 min., and the supernatant was then passed

sequentially through 0.8, 0.45, and 0.2 micron filters. The extract was run on the HCGβ affinity column and recirculated overnight, and eluted as before. Silver-stained SDS-PAGE results were consistent with prior results on callus-expressed HCGβ. ELISA quantification indicated that 300 μg HCGβ had been purified from the pDAS920 plants.

One larger plant without roots was collected from an event which had been transformed with pDAS940, tested positive for HCGβ expression in callus and in the plant regenerated from the callus. It also was positive in the Northern analysis for GalT expression at the callus stage. A 50 gm sample of frozen plant tissue was divided into two parts each of about 25 grams. To each part was added 200 ml of extraction buffer as before. The mixture was pulverized on a Bead-Beater® with 1 mm Zirconia® beads as before, and passed through a glass filter and then filtered through 0.8, 0.45 and 0.2 micron filters without centrifugation. The clarified extract was loaded and recirculated overnight on the affinity column, and eluted as described earlier. Silver-stained SDS-PAGE results were consistent with prior results from callus-expressed HCGβ. ELISA quantification indicated that about 55 μg HCGβ had been purified from the pDAS940 plant tissue.

13. <u>Biochemical Analysis of Transformed Callus and Plants.</u> The following table provides the samples of affinity-purified maize-expressed HCGβ from callus and plants that were analyzed for protein glycosylation.

Summary of samples of purified HCGβ.

Description	**Amount of Protein**
HCGβ-GalT prepared from transformed maize callus in August 2003.	~ 200 μg
HCGβ-only prepared from transformed maize callus in August 2003.	~ 1,000 μg
HCGβ- GalT prepared from transformed maize callus in October 2003.	~ 8 μg
HCGβ-only prepared from transformed maize callus in October 2003.	~ 1,000 μg
HCGβ- GalT prepared from transformed maize plant in October 2003.	~ 52 μg
HCGβ-only prepared from transformed maize plant in October 2003.	~ 200 μg

14. Protein reduction, Carboxymethylation, and Tryptic digestion. 105 μL (~ 300 μg) of HCGβ-only samples (event pDAS920, callus-expressed) was aliquoted in each experiment. The full amounts of the other samples were used for analyses. Each affinity-purified protein sample was dried in a centrifugal evaporator in a microcentrifuge tube (0.6 mL, siliconized). The pellet was resuspended in 180 μL of protein dissolution buffer (6M Guanidine hydrochloride/ 0.4M ammonium bicarbonate, pH7.8). The samples were reduced by addition of 10 μL of 0.1M DTT and incubated at 65° C for 1 hr. After reduction, the protein samples were alkylated by addition of 20 μL of 0.2M iodoacetamide (IAA) and incubated at room temperature for 1 hour in the dark. Alkylation reaction was quenched by addition of 40 μL of 0.1M DTT and incubated at room temperature for 15 min. The protein was then desalted using a reversed phase cartridge (Protein Macro Trap, Michrom Bioresources, cat. no. 004-25108-53) according to the manufacturer's procedure and eluted first with 200 μL of 80% acetonitrile/0.1% TFA, followed by 200 μL of 100% acetonitrile/0.1% TFA and the eluted protein was dried in a centrifugal evaporator. The desalted reduced/alkylated protein was resuspended in 100 μL of digestion buffer (100 mM Tris-HCl, pH 8.5) and solution of trypsin (sequencing grade, Roche, cat no. 1-418-025) was added (80 μL; prepared by dissolution of 25 μg of trypsin in 0.5 mL of 25 mM ammonium bicarbonate buffer). The sample was incubated for 16 hrs at 37° C. In case when HPLC was performed (*i.e.*, the two August samples), the digests were concentrated to approximately 220 μL by centrifugal evaporation. The tryptic digests were stored at -20° C until further steps were performed.

15. HPLC Fractionation of the Tryptic Digest. HPLC fractionation of the tryptic digest was performed for the two August samples in order to resolve two possible N-glycosylation sites and increase sensitivity for glycopeptide detection. A Luna C18 4.6 mm ID x 150 mm length (Phenomenex, cat. no. 00F-4252-E0-L) column and an Agilent 1100 LC system were used for the separation. Constant flow rate of 2 mL/min was used for the separation. 200 μL of the tryptic digest mixture was injected. The separation of peptides was accomplished using the following gradient: 100% solvent A (3% acetonitrile/ 0.06% TFA) isocratic for 2 min, 0 to 10% solvent B (80% acetonitrile/ 0.05% TFA) in 2 min, 10 to 40% solvent B in 19 min, 40 to 100 % solvent B in 5 min, then 100% solvent B isocratic for 5 min. The column was then re-equilibrated in solvent A (0 to 100% solvent A in 2 min), and washed with 100% solvent A for 3

min. The separation was performed at room temperature. Elution of peptides was monitored by UV absorption at 205 nm. A Gilson FC-203B fraction collector was used to collect 80 1-mL fractions (in siliconized microcentrifuge tubes) (0 to 40 min). The collected fractions were dried in a centrifugal evaporator following the separation. Fractions were re-suspended in 4 μL of 50% acetonitrile/0.1% TFA and 1 μL of the material in each fraction was examined by MALDI MS.

16. **Enzymatic Deglycosylation (PNGase-A Procedure).** For the two August samples, tryptic glycopeptides isolated by HPLC (combined fractions 20 and 21) were treated with peptide-N-glycosidase A (from almonds) solution ("PNGase A", Roche, cat no. 1 642 995). For the rest of the samples, the whole tryptic digest (after desalting on a C18 cartridge (Peptide Macro Trap, Michrom Bioresources, cat no. 004-25108-52, as per standard protocol) was treated with PNGase-A. Peptides were dried in a centrifugal evaporator, and re-dissolved in 10 μL of 20 mM ammonium acetate buffer, pH 5.0. Ten microliters of PNGase-A were added to the samples. The samples were incubated at 37° C for 16 hours.

17. **Purification of Released Oligosaccharides.** The proteolytic/PNGase-A digest was passed through C18 cartridge (Peptide Macro Trap, Michrom Bioresources, cat no. 004-25108-52, pre-conditioned according to manufacturer's procedure) and the flow-through fraction was collected. The cartridge was washed with 0.2 mL of 0.1% aqueous TFA and the wash was combined with the first flow-through fraction. The C18 cartridge was regenerated by 0.5 mL of 100% acetonitrile/0.1% TFA (deglycosylated peptides were collected and analyzed by MALDI MS at this step), then re-equilibrated with 0.5 mL of 0.1% aqueous TFA. The flow-through fraction from the first passage was passed through the C18 cartridge again. The resulting flow-through fraction, containing released oligosaccharides, was further purified using a porous graphitic carbon cartridge (E-cartridge, QA-Bio, cat no. C-E001) according to the manufacturer's procedure. Oligosaccharides were eluted from the E-cartridge with 50% acetonitrile/0.1% TFA and dried to completeness in a centrifugal evaporator. The glycan samples were re-dissolved in 3 μL of high-purity Milli-Q water (18 mega-ohm cm, TOC 21 ppb) and passed through C18 ZipTips (Millipore), pre-conditioned according to the manufacturer's procedure. 50% of the purified glycan sample solutions were deposited onto MALDI sample plate, overlaid with sDHB

matrix, and dried. The corresponding MALDI spectra were recorded as described below. The other 50% of the purified glycan sample solutions were used for treatment with β-galactosidase.

18. **Treatment of Free N-Glycans with β-galactosidase.** 50% of the purified glycan sample solutions were treated with β(1-3,4)-galactosidase from Bovine Testes in order to determine which N-glycans in the total N-glycan pool of each sample contain terminal β(1-3,4)-galactose residue. The N-glycans in each sample were dried in a centrifugal evaporator, and re-dissolved in 14 μL of Milli-Q deionized water. Next, 4 μL of 5x reaction buffer (QA-Bio, Lot no. D304.1) and 3 μL of β-galactosidase solution (QA-Bio, cat. no. E-BG02, Lot no. D304.1) were added to the sample. The samples were incubated at 37° C for 16 hours. After β-galactosidase treatment, N-glycans were again purified as described above, and the corresponding MALDI spectra were recorded.

19. **MALDI-TOF MS and MALDI-PSD.** Voyager DE-STR (Applied BioSystems, serial no. 4260) MALDI-TOF mass spectrometer operated in positive reflectron mode was used to obtain data for peptides and oligosaccharides.

The following settings were used to obtain MALDI spectra of peptides. The acceleration voltage was set to 20 kV. The grid voltage was set to 66% of the acceleration voltage. The delay time varied between 215 and 300 nsec. The laser setting varied between 2500 and 3000. A summation of 500 acquisitions was averaged in each spectrum. The mass scale was calibrated with the following standard peptides (Applied BioSystems): des-Arg^1-Bradykinin, m/z 904.4; Angiotensin I, m/z 1,296.6; Glu^1-Fibrinopeptide B, m/z 1570.6; Neurotensin, m/z 1672.9; ACTH (clip 1-17), m/z 2093.0; ACTH (clip 18-39), m/z 2465.1; ACTH (clip 7-38), m/z 5730.6. A 1 μL sample of purified peptides was deposited onto a MALDI sample plate, overlaid with 1 μL of CHCA matrix (α-cyano-hydroxycinnamic acid) and air-dried. MALDI-PSD spectra were recorded using mirror voltage ratio 1.12 and the following mirror ratios: 1, 0.85, 0.75, 0.65, 0.55, 0.4, 0.3, 0.2, 0.1, 0.05.

The following settings were used to obtain MALDI spectra of free oligosaccharides (glycans). The acceleration voltage was set to 20 kV. The grid voltage was set to 69% of the acceleration voltage. The delay time was set to 215 nsec. The laser setting was approximately 3500. A summation of 500 acquisitions was averaged in each spectrum. The mass scale was calibrated with the following standard oligosaccharides: $(GlcNAc)_2(Man)_5$, m/z (MNa^+) = 1257.46; $(GlcNAc)_4(Man)_3(Fuc)$, m/z (MNa^+) = 1485.56; $(Gal)(GlcNAc)_4(Man)_3(Fuc)$, m/z ($MNa^+$) = 1647.62; $(Gal)_2(GlcNAc)_4(Man)_3(Fuc)$, m/z ($MNa^+$) = 1809.68. A 2 μL sample of purified glycans was deposited onto a MALDI sample plate, let to almost dry, then overlaid with 1 μL of sDHB matrix (9:1 v/v mixture of 18 mg/mL 2,5-dihydroxybenzoic acid in 66% acetonitrile and 15 mg/mL 2-hydroxy-5-methoxybenzoic acid in 66% acetonitrile) and air-dried.

MALDI-TOF MS and MALDI-PSD data were analyzed using Data Explorer v4.0 software (Applied BioSystems). Molecular weights and amino acid sequences of peptides and glycopeptides were attributed to the sequence of HCGβ using MassLynx v3.4 software (Micromass). The software tool for oligosaccharide mass-spectrometry analysis developed at the Dow Chemical Company was used to interpret mass-spectra of the glycan samples.

20. **<u>Results.</u>** N-glycan profiles of HCGβ-only and HCGβ-GalT samples obtained from maize callus and whole plants were analyzed and compared to each other. The corresponding N-glycan samples were investigated for the presence of β-galactosylated chemical structures.

Chromatographic profiles of the tryptic digests for the August samples of HCGβ-GalT and HCGβ-only were very similar to each other as shown in <u>Exhibit D</u>. Tryptic fragments containing Asn33 glycosylation site (T3 fragment) and its glycoforms were detected in both HCGβ-containing samples. Identity of the T3 (containing Asn33) fragment was confirmed by MALDI PSD after enzymatic deglycosylation (data not shown). The fragment containing the other glycosylation site (Asn50; T4 fragment) was not detected in either sample.

Profiles of glycopeptides were found to be very similar for both the HCGβ-GalT and HCGβ-only callus-expressed HCGβ samples as shown in <u>Exhibit E.</u> Profiles of free N-glycans were also

found to be very similar for both the HCGβ-GalT and HCGβ-only callus-expressed HCGβ samples as shown in Exhibit F. The corresponding summaries of patterns of the N-glycans released from HCGβ-GalT and HCGβ-only callus-expressed samples are shown in Exhibits G and H (August samples) and in Exhibits I and J (October samples). The major two glycan structures in all of these HCGβ samples were $(HexNAc)_2(Hex)_3Xyl$ (or N2H3X) and $(HexNAc)_2(Hex)_3(Xyl)(Fuc)$ (or N2H3XF), which are typical for plant-derived glycoproteins.

Similar N-glycan profiles were observed for plant-expressed HCGβ-GalT and HCGβ-only samples as shown in Exhibit K (MALDI mass-spectra of free N-glycans) and Exhibits L and M (corresponding data summaries).

The table provided in Exhibit N provides the amount of each observed N-glycan in each sample in a side-by-side comparison. Minor differences in the glycan profiles were observed. These differences were closely examined before and after treatment of the samples with β-galactosidase (enzyme that removes terminal β-Gal residues). Results of the β-galactosidase treatment experiment, combined with consideration that initial galactosylated N-glycans must contain at least three GlcNAc and at least four Hex residues, demonstrate that there is only one difference between the N-glycan profiles of the HCGβ-GalT and HCGβ-only samples that can be attributed to galactosylation by β1,4-galactosyltransferase: a glycan with composition $(GlcNAc)_3(Hex)_6$ (or N3H6; theor. m/z 1622.60).

The figures in Exhibit O and Exhibit P show the results of β-Gal removal from the N3H6 glycan of maize callus-expressed and plant-expressed HCGβ-GalT, respectively. As shown, upon β-Gal removal, relative intensity of the N3H6 glycan peak decreases, while relative peak intensity of the resulting de-galactosylated glycan N3H5 increases. The figure in Exhibit Q shows removal of β-Gal from a tetra-galactosylated glycan standard in a control experiment. Because only one Gal residue is removed from N3H6 glycan by β-galactosidase, the structure of the N3H6 glycan is likely to be
$(\beta 1,4\text{-}Gal)(GlcNAc)(Man)_2(Man)_3(GlcNAc)_2$. This is a minor glycan in the total glycan mixture. In callus-expressed HCGβ-GalT sample, N3H6 glycan was detected at 1-2% level; in plant-

expressed HCGβ-GalT sample, it was detected at only 0.5% level. See the tables in Exhibits G, I, L and N.

21. Summary and Conclusions. A gene for the β-chain of human chorionic gonadotropin (HCGβ), a model glycoprotein, was introduced into maize callus. Two constructs were introduced into the callus: one expressing HCGβ only, and another one co-expressing HCGβ with human β1,4-galactosyltransferase-I (GalT-I). The transformed callus was regenerated into transformed maize plants. The maize-expressed HCGβ samples were affinity-purified from the corresponding maize callus and leaf tissue and the distributions of N-linked glycans in the HCGβ samples were analyzed.

The two major glycan structures in the HCGβ samples were (HexNAc)2(Hex)3Xyl (or N2H3X) and (HexNAc)2(Hex)3(Xyl)(Fuc) (or N2H3XF), which are typical for plant-derived glycoproteins. Based on β-galactosidase treatment experiments, the major N-glycans N2H3X and N2H3XF do not contain galactose (data not shown). Absence of galactose in N2H3X and N2H3XF N-glycans is also supported by the knowledge that N-linked glycans possess a conserved trimannosyl core N2M3 (where M is mannose residue (a hexose), and N is GlcNAc residue). Minor differences in the glycan profiles were observed between HCGβ-only and HCGβ-GalT samples. These differences were closely examined before and after treatment of the samples with β-galactosidase. Results of these experiments demonstrated that there was only one difference between the N-glycan profiles of the HCGβ-only and HCGβ-GalT samples that can be attributed to galactosylation by β1,4-galactosyltransferase: a glycan with composition (GlcNAc)3(Hex)6 (or N3H6). Because only one Gal residue was removed from N3H6 glycan by β-galactosidase, the structure of the N3H6 glycan is most likely (β1,4-Gal)(GlcNAc)(Man)2(Man)3(GlcNAc)2. This N3H6 glycan structure does not contain fucose or xylose. In the callus-expressed HCGβ-GalT sample, N3H6 glycan was detected at a 1-2% level; while in the plant-expressed HCGβ-GalT sample, it was detected at a 0.5% level. The relative abundance and/or total amount of galactosylated N-linked glycan structures of the exogenous glycoprotein that is obtained per cell, callus and/or plant is expected to be optimized for commercial production.

The experiments discussed in this affidavit demonstrate the successful placement of galactose on N-linked glycan structures of human chorionic gonadotropin (HCGβ) in Whiskers®-transformed corn cells and corn plants expressing human β1,4-galactosyltransferase (GalT) (*i.e.*, a glycosyltransferase) and HCGβ (*i.e.,* an exogenous glycoprotein), both of which expressions were driven by the maize ubiquitin 1 promoter. Thus, these additional experiments further support the disclosure and claims in WO 00/34490 for methods of using transformed plant cells and transformed whole plants to produce (*i.e.*, manufacture) a glycoprotein having a human-type sugar chain.

I, William Henry Kerr Anderson, certify, attest and swear that the foregoing statements are correct and complete.

Dated: Nov 20 2003 Signed: W H K Anderson

Subscribed and sworn before me on this 20th day of November, 2003.

Melissa A. Wagner
Notary

Melissa A. Wagner
Notary Public, Bay County, Michigan
Acting In Midland County, Michigan
My Commission Expires May 08, 2006

Seal

CURRICULUM VITAE

W. H. KERR ANDERSON, B.Sc., Ph.D.

ACADEMIC HISTORY

School:	University of Aberdeen, Scotland
Degree:	Ph.D.
Major:	Biochemistry/Immunochemistry
Graduated:	1976
Thesis:	Purification and Characterization of C1s-Inhibitor

School:	University of Aberdeen, Scotland
Degree:	B.Sc. (with Honors)
Major:	Biochemistry
Graduated:	1973

EMPLOYMENT HISTORY

1999-Present Dow Chemical - Technical Director and Scientist for Plant Biopharmaceuticals: I am responsible for Research and Development of systems expressing human pharmaceutical proteins in plants, leveraging resources broadly across Dow and its subsidiaries and with external parties. I also participate in customer presentations and am responsible for public presentations at technical meetings. I am currently the senior ranking Scientist in Biotechnology at Dow.

1997-1999 Dow Chemical - Leader, External Technology: Part of a team evaluating biotechnology opportunities for Dow. I was directly responsible for the External Technology component of this work, identifying resources at Universities and other companies which could be leveraged.

1995-1997: Dow Chemical - Technical Leader, Time-temperature Indicator Project: Part of a team responsible for the development and assessment of an opportunity in the food and pharmaceutical industry

1993-1995: Dow Chemical -Group Leader: Part of team assigned to assess new business opportunities for biotechnology for Dow, focussed primarily on evaluation of antibody-based technologies in a new analytical environmental business platform.

1991-1993: Dow Chemical - Group Leader, Bioproducts Laboratory Central R&D: Managed research group focussing on wide range of early stage projects -

- Production of genetically engineered fragments and single chain variants of murine antibodies
- Development of novel methods for the identification of tumor specific antibodies
- Development of technology to identify extremely rare high producer variants of antibody producing mammalian lines.
- Development of PEG conjugation of proteins as an approach to the reduction of cross-species immunogenicity
- Development of monoclonal antibodies specific for HLA-DR determinants and molecular variants of HLA-DR and T cell receptors associated with rheumatoid arthritis
- Development of an antibody-based analytical procedure for the anti-microbial DBNPA

1981-1983:	Assistant Professor Department of Microbiology and Immunology Tulane University School of Medicine, New Orleans LA
1978-1981:	Research Associate with Dr. Max Cooper and Dr. John Kearney Lurleen B Wallace Comprehensive Cancer Care Center University of Alabama at Birmingham AL
1976-1978:	Post-Doctoral Fellow with Dr. R.M Stroud Department of Microbiology University of Alabama at Birmingham AL

PUBLICATIONS

Papers:

1 Fothergill, J.E,. Anderson, W.H.K., (1978) A molecular approach to the complement system. Current Topics in Cellular Regulation. **13,** 259-311,

2 Anderson, W.H.K., Stroud, R.M., (1979) Generation and enrichment of C4d in whole serum. Journal of Immunological Methods. **29**, 323-30

3 Burckhardt, J.J., Anderson, W.H.K., Kearney, J.F., Cooper, MD, (1982) Human blood monocytes and platelets share a cell surface component. Blood **60**, 767-71

4 Lammie, P.J., Katz, S. P., Anderson, W.H.K., (1984) Serosuppression in experimental filariasis. Clinical & Experimental Immunology **55**, 602-10

5 Anderson, W.H.K., Reed, J. A. and Pollock, D.K. (1993) Elicitation of a predominantly lambda light chain-bearing antibody response in BALB/c mice to a novel bifunctional aminocarboxylate chelating agent. Hybridoma **12**, 677-688

6 Slavin-Chiorini, D.C. Kashmiri, S.V. Schlom, J. Calvo, B. Shu, L.M. Schott, M. E. Milenic, D.E. Snoy, P. Carrasquillo, J. Anderson, K. (1995) Biological properties of chimeric domain-deleted anticarcinoma immunoglobulins Cancer Research. **55 (23 Suppl)**, 5957s-5967s

Patents:

1 US Patent 5,993,813 Family of high affinity modified antibodies for cancer treatment

2 US Patent 6,051,225 Family of high affinity modified antibodies for cancer treatment

3 US Patent 6,207,815 Family of high affinity modified antibodies for cancer treatment

4 US Patent 6,333,405 High affinity humanized anti-CEA monoclonal antibodies

5 US Patent 6,348,581 High affinity humanized anti-TAG-72 monoclonal antibodies

6 US Patent 6,417,337 High affinity humanized anti-CEA monoclonal antibodies

7 US Patent 6,641,999 Probing Method for Identifying Antibodies Specific for Selected Antigens

8 US publication 20030004318 High Affinity Humanized Anti-Tag-72 Monoclonal Antibodies

9 US publication 20030013856 High Affinity Humanized Anti-Tag-72 Monoclonal Antibodies

10 US publication 20020193574 High Affinity Humanized Anti-Tag-72 Monoclonal Antibodies

11 US publication 20020183497 High Affinity Humanized Anti-Tag-72 Monoclonal Antibodies

12 US publication 20030013854 High Affinity Humanized Anti-Tag-72 Monoclonal Antibodies

13 US publication 20030018171 High Affinity Humanized Anti-CEA Monoclonal Antibodies

14 US publication 20030027994 High Affinity Humanized Anti-CEA Monoclonal Antibodies

15 US publication 20020165387. High Affinity Humanized Anti CEA Monoclonal Antibodies

16 US publication 20020183495 High Affinity Humanized Anti CEA Monoclonal Antibodies

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1 Anderson, W.H.K., Fordyce, W. A., Pollock, D.K. (1991) Discrimination between geometric isomers of Rhodium-BA-2,3,2,TET by monoclonal antibodies. J. Nucl. Med. **32**, 914-915

2 Anderson, W.H.K., Goeckeler, W.F., Harrington, C.K., Spittka, G. A., Stoneburner, L.K. and Verbanac, K.M. (1991) Modification of the biodistribution of radiolabelled CC49 in tumor-bearing mice with anti-idiotypic antibodies. J. Nucl. Med. **32**, 1058

3 Depalatis, L.R., Coleman, J.S., Anderson, W.H.K., Kaplan, D. A., Spittka, G. A. (1991) The CX-1 model: biodistribution profile, tumor autoradiography of I-125 labeled IgG, $F(ab')_2$ and Fab forms of monoclonal antibody CC49 and correlative immunohistochemical localization of TAG-72 antigen. J. Nucl. Med. **32**, 160

4 Mezes, P. S., Gourlie, B.B., Johnson, K.A., Anderson, W.H.K., Richard, R.A., Kaplan, D. A. (1991) Generation of an anti-tumor associated glycoprotein-72 (TAG-72) antibody with a human light chain. Second Annual Antibody Engineering Meeting, San Diego CA

5 Rixon, M.W., Anderson, W.H.K., Gourlie, B.B., Kan, L., Mezes, P. S., Kaplan, D. A. (1991) Preferential utilization of a heavy chain variable region in TAG-72 binding antibodies. Second Annual Antibody Engineering Meeting, San Diego CA

6 Gourlie, B.B., Mezes, P. S., Rixon, M.W., Anderson, W.H.K., Johnson, K.A., Goeckeler, W.F. (1991) Preparation and characterization of chimeric anti-tumor antibodies CC49 and CC83. Second Annual Antibody Engineering Meeting, San Diego CA

Invited Presentations on Plant-made Pharmaceuticals:

1 BIO 2001 Conference, June 2001, San Diego CA

2 Antibody Production and Downstream Processing, Feb 13-15 2002, San Diego CA

3 Waterside Conference, May 19-22 2002, Savannah GA

4 Wilbio Conference on Transgenic Bioprocessing, May 23-24 2002, Savannah GA

5 BIO 2002 Conference, June 2002, Toronto Canada

6 Antibody Manufacturing and Production, September 19-20 2002, Long Beach, NJ

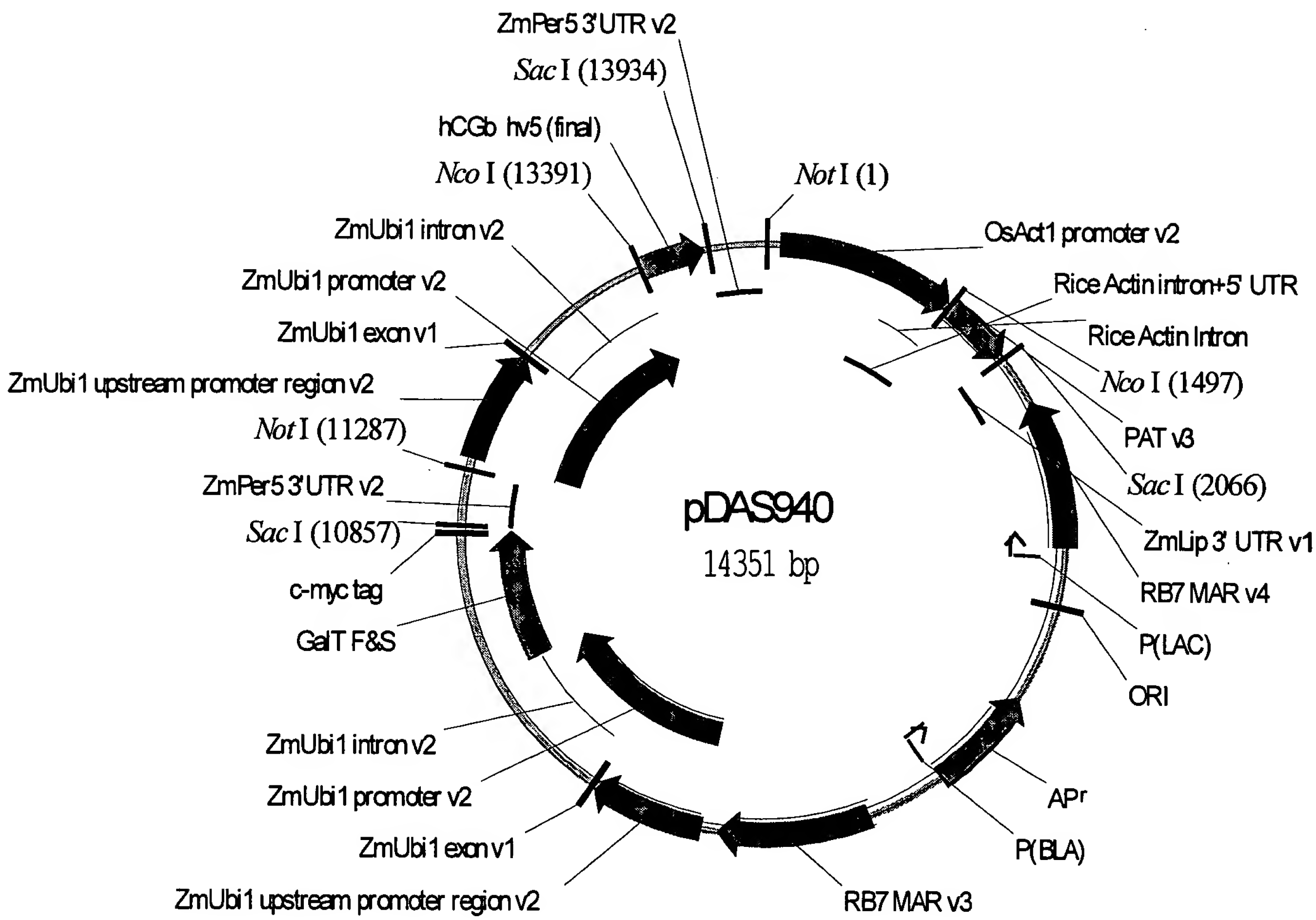
7 Waterside Conference Process Development and Production Issues for Monoclonal and Recombinant Antibodies, May 5-7 2003 San Francisco

8 Wilbio Conference on Transgenic Bioprocessing, May 8-9 2003, San Francisco CA

9 BIO 2003 Conference June 23-25 2003, Washington DC

10 Plant-made Pharmaceuticals Dec 8-9 2003, Arlington VA

Exhibit A



ZmPer5 3'UTR v2
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hCGb hv5 (final)
Nco I (13391)
Not I (1)
ZmUbi1 intron v2
OsAct1 promoter v2
ZmUbi1 promoter v2
Rice Actin intron+5' UTR
ZmUbi1 exon v1
Rice Actin Intron
ZmUbi1 upstream promoter region v2
Nco I (1497)
Not I (11287)
PAT v3
Sac I (2066)
ZmPer5 3'UTR v2
pDAS940
14351 bp
Sac I (10857)
ZmLip 3' UTR v1
c-myc tag
RB7 MAR v4
GalT F&S
P(LAC)
ORI
ZmUbi1 intron v2
APr
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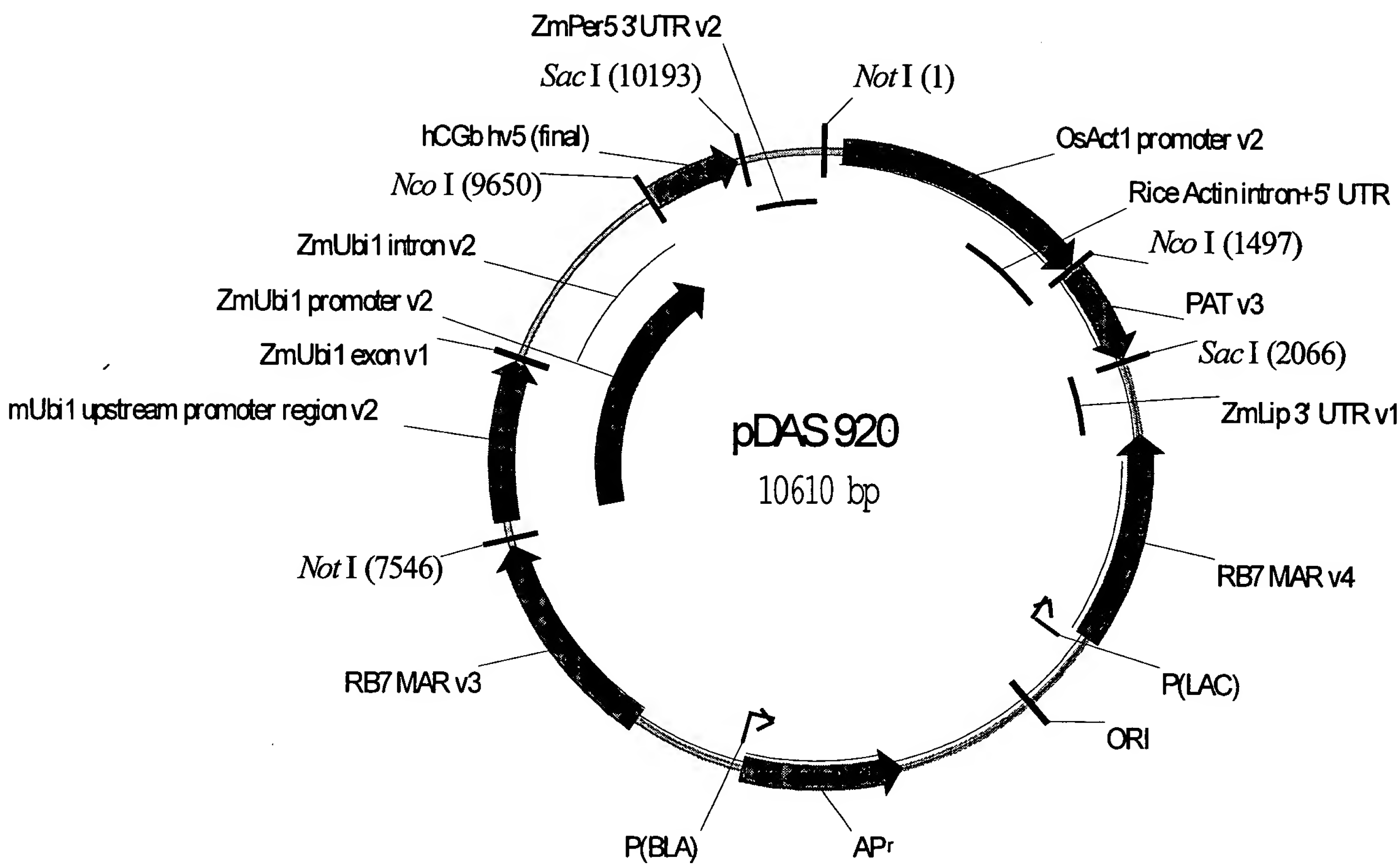
RB7 MAR v3

Exhibit B

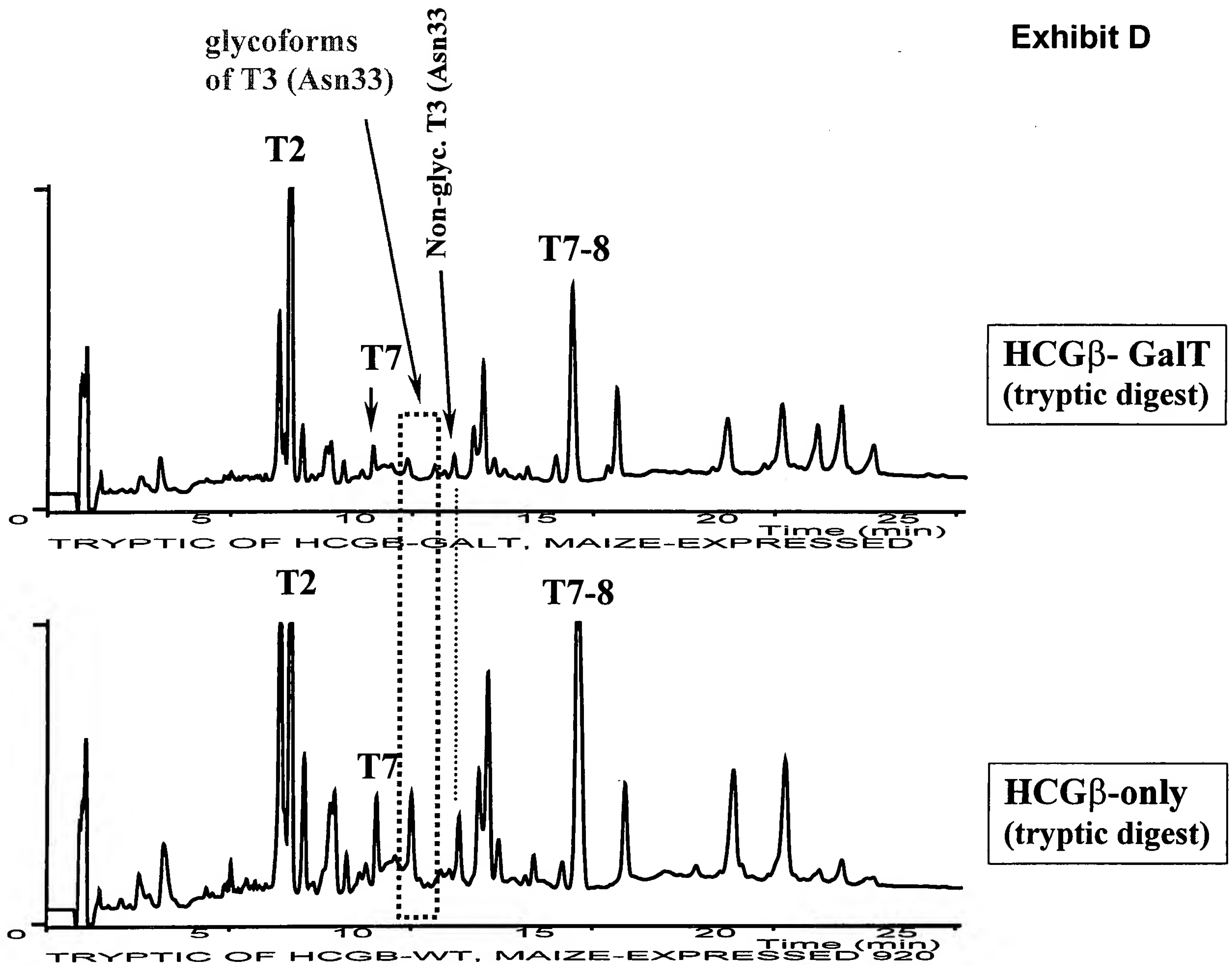


ZmPer5 3'UTR v2
Sac I (10193)
Not I (1)
hCGb hv5 (final)
OsAct1 promoter v2
Nco I (9650)
Rice Actin intron+5' UTR
Nco I (1497)
ZmUbi1 intron v2
ZmUbi1 promoter v2
PAT v3
ZmUbi1 exon v1
Sac I (2066)
mUbi1 upstream promoter region v2
ZmLip 3' UTR v1
pDAS 920
10610 bp
Not I (7546)
RB7 MAR v4
RB7 MAR v3
P(LAC)
ORI
P(BLA)
APr

Exhibit C

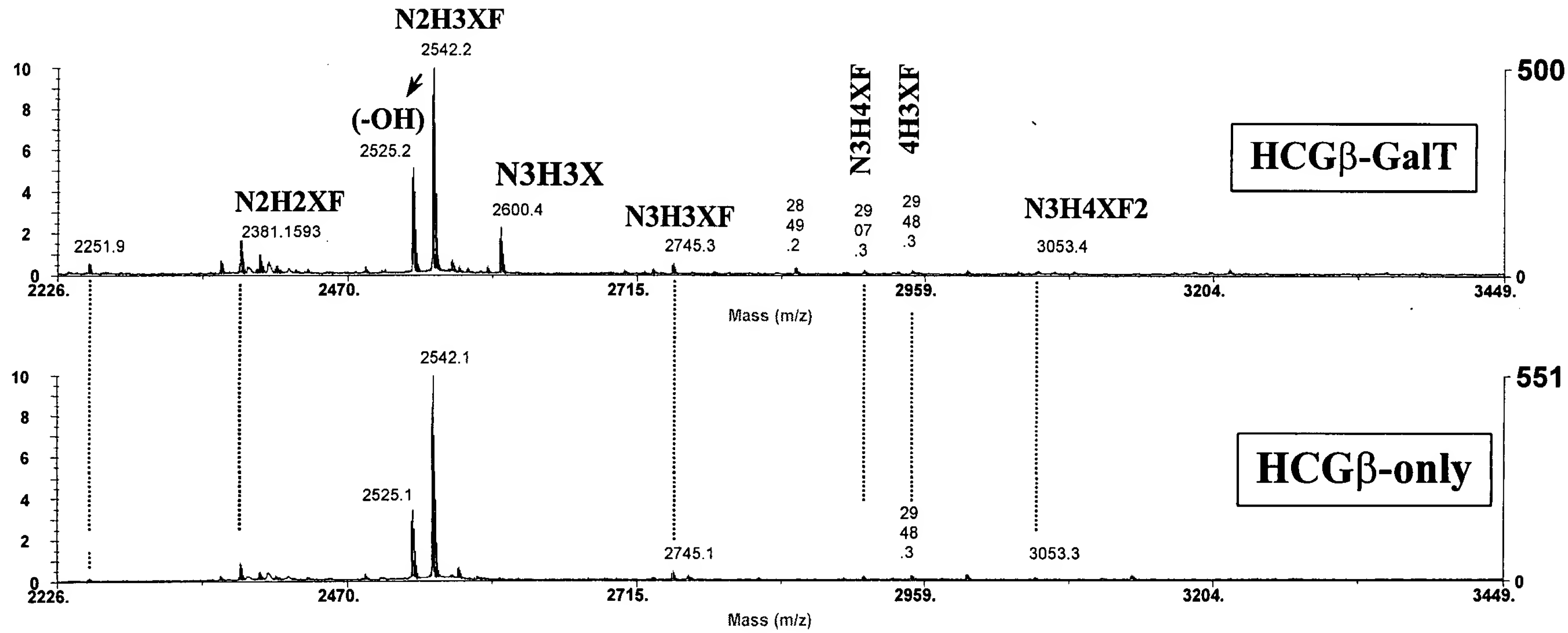
A-run	AAAA	
A-Trun	WWWWWW	
AU destab	ATTTA	Shaw, G. & R. Kamen, Cell 46:659-667 (1986)
BEAF-32	CGATA	Zhao et al., Cell 81:879-889 (1995)
Bt poison	TTAATTAACCAAAGAATAGA	from Monsanto patent US 5,500,635
C-run	CCCC	
DST	GGAGNNNNNCATAGATTGN	Saur mRNAs: Newman, T.C. et al., Plant Cell 5:701-714 (1993)
G-run	GGGG	
hairpin	CTTCGG	Turek, C., et al., PNAS 85:1364-1368 (1988)
hairpin cmp	CCGAAG	complement of the hairpin
homeobox	ATTATTACATGNG	Freeling, Lubkowitz and Bauer. PCT WO 2001075071 A1
homeobox	ATTATTATTACATGNG	Freeling, Lubkowitz, and Bauer. PCT WO 2001075071
plant 3'spl	TGCAGG	EMBO J. 10:2635 (1991)
plant 5'spl	AGGTAAGT	EMBO J. 10:2635 (1991); Nucl. Acids Res. 14:9549 (1986)
poly A	AATAAA	Nucl. Acids Res. 15:9627 (1987) and Adang
poly A	AATAAG	Adang 5,380,831 (Jan 10, 1995)
poly A	GATAAA	Adang 5,380,831
poly A	AATATT	Adang 5,380,831
poly A	AATGAA	Adang 5,380,831
poly A	CATAAA	Monsanto Patent Application (WO 90/10076, Sept 7, 1990)
poly A	AATACA	Monsanto Patent Application (WO 90/10076, Sept 7, 1990)
poly A	AATTAA	Monsanto Patent Application (WO 90/10076, Sept 7, 1990)
poly A	ATTAAA	Monsanto Patent Application (WO 90/10076, Sept 7, 1990)
poly A	AAAATA	Monsanto Patent Application (WO 90/10076, Sept 7, 1990)
poly A	ATACAT	Monsanto Patent Application (WO 90/10076, Sept 7, 1990)
poly A	ATTAAT	Monsanto Patent Application (WO 90/10076, Sept 7, 1990)
poly A	AAGCAT	Monsanto Patent Application (WO 90/10076, Sept 7, 1990)
poly A	ATGAAA	Monsanto Patent Application (WO 90/10076, Sept 7, 1990)
poly A	ATAAAA	Monsanto Patent Application (WO 90/10076, Sept 7, 1990)
poly A	ATACTA	Monsanto Patent Application (WO 90/10076, Sept 7, 1990)
poly A	AATCAA	Monsanto Patent Application (WO 90/10076, Sept 7, 1990)
poly A	ATATAA	Monsanto Patent Application (WO 90/10076, Sept 7, 1990)
poly A	AACCAA	Monsanto Patent Application (WO 90/10076, Sept 7, 1990)
poly A	AATAAT	Adang 5,380,831 and Monsanto Patent Application (WO 90/10076,
RNAPII term	CANNNNNNNNNAGTNNAA	see below
RNAPII term	CANNNNNNNNNNNAGTNNAA	see below
RNAPII term	CANNNNNNNNAGTNNAA	Vankan, P., & W. Filipowicz, EMBO J. 7:791-799 (1988)
T-run	TTTT	
Unwinding	AATATATTT	Bode et al., Int. Rev. Cytol. 162A 389-454 (1995)

Exhibit D



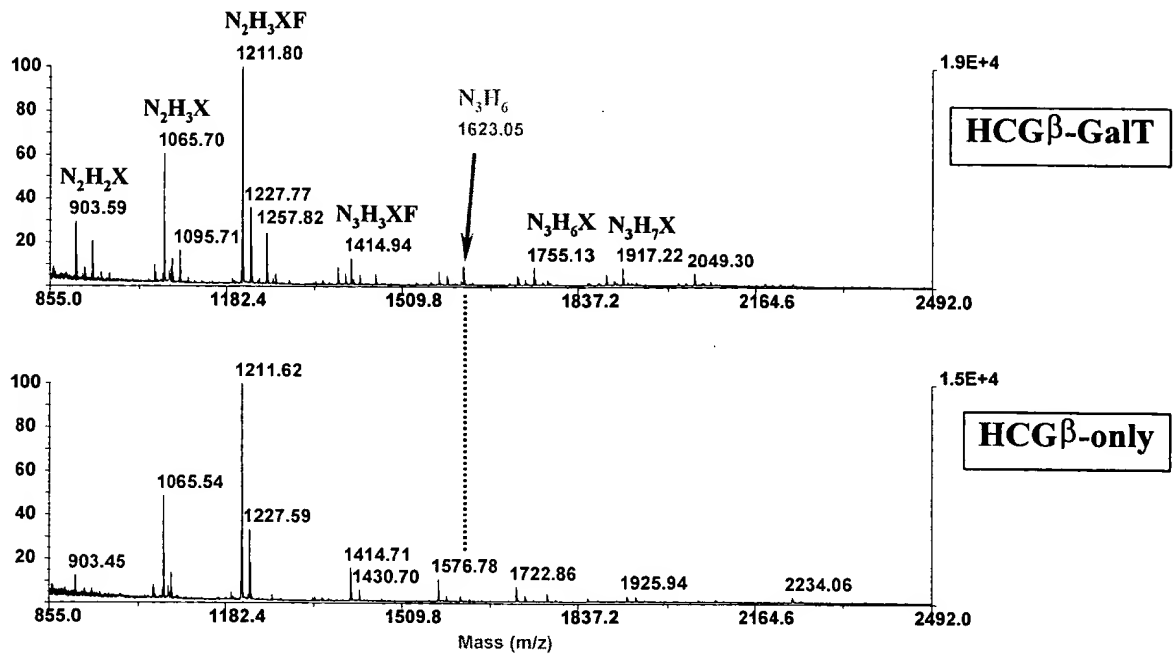
Callus, August Samples. Chromatograms representing separation of HCGβ tryptic fragments. Upper panel: HCGβ-GalT tryptic digest. Lower panel: HCGβ-only tryptic digest. Boxed area shows the region of the chromatograms where glycoforms of T3 fragment (containing Asn33 glycosylation site) were detected (HPLC fractions 20 and 21). Non-glycosylated form of T3 fragment eluted in a later HPLC fraction (fraction 23).

Exhibit E



Callus, August Samples. Representative MALDI mass-spectra of glycoforms (glycopeptides) of T3 fragment (containing Asn33 glycosylation site). The assignment for the most intense peaks is given in the figure (only the glycan part). H = hexose (mannose or galactose), N = HexNAc, F = fucose, X = xylose. Upper panel: HCGβ-GalT. Lower panel: HCGβ-only. The corresponding HPLC chromatograms are shown in Exhibit D.

Exhibit F



Callus, October Samples. A comparison of representative MALDI MS profiles of N-glycans released form callus-expressed HCGβ-GalT (upper panel) and HCGβ-only (lower panel) samples. Assignments for some m/z peaks are shown in the figure. M/z 1623 peak (assigned to N3H6 glycan) has noticeable intensity (~2.0% of the sum of peak intensities) in HCGβ-GalT sample, whereas it is absent in HCGβ-only sample. M/z 1623 glycan peak is not the only difference between the HCGβ-GalT and HCGβ-only glycan profiles, but m/z 1623 glycan is the only one that loses galactose residue upon β(1-3,4)-galactosidase treatment.

Glycoforms observed on Asn33 site of HCGβ-GalT (callus, August sample).

N-Glycan	H-T3 glycopeptide m/z theor.	H-T3 glycopeptide m/z obs.	Comment	Corresponding released free glycan m/z theor.	Corresponding released free glycan m/z obs.	% of total intensity in MALDI of free glycans	Comment
no glycans	1372.73	1371.78	sequence of deglycosylated peptide was confirmed by PSD	n/a	n/a		
N	1575.81	ND	ND	n/a	n/a		
N2	1778.89	ND	ND	n/a	n/a		
N2H2X	2235.07	2235.08	trace	903.32	903.42	6.1	
N2H3	2265.09	ND		933.34	933.43	trace	
N2H2XF	2381.13	2380.16	significant signal	1049.38	ND	0.0	
N2H3X	2397.13	2396.16	***	1065.38	1065.49	22.7	
N2H4	2427.15	2426.20	*	1095.40	1095.51	2.7	
N2H3XF	2543.19	2542.21	major	1211.44	1211.57	24.7	
N2H4X	2559.19	2558.22	***	1227.44	1227.55	8.9	overlapping with K-adduct of N2H3XF
N2H5	2589.21	2588.25	**	1257.46	1257.58	6.2	
N3H3X	2600.21	2599.41	significant signal	1268.46	ND	0.0	
N2H5X	2721.25	2721.31	trace	1389.50	1389.64	3.3	
N2H5F	2735.27	ND	ND	1403.52	1403.66	trace	
N3H3XF	2746.27	2745.33	***	1414.52	1414.67	7.4	
N3H4X	2762.27	ND	ND	1430.52	1430.66	1.0	
N3H5	2792.29	ND		1460.54	1460.67	1.0	
N3H4X2	2894.32	2893.36	*	1562.57	1562.73	1.6	only tentative assignment
N3H4XF	2908.33	2907.38	**	1576.58	1576.73	2.9	
N3H6	2954.35	2954.36	trace	1622.60	1622.75	1.1	
N3H4X2F	3040.37	3039.43	*	1708.62	1708.79	1.0	only tentative assignment
N3H5X2	3056.38	3055.49	*	1724.63	1724.78	2.3	only tentative assignment
N3H6X	3086.40	3085.38	*	1754.65	1754.78	2.3	
N3H5X3	3188.42	3187.53	*	1856.67	1856.82	1.2	only tentative assignment
N3H6X2	3218.44	3217.48	**	1886.69	1886.84	2.1	only tentative assignment
N3H7X	3248.46	3247.46	trace	1916.71	1916.85	0.8	
N3H6X3	3350.48	3350.48	*	2018.73	2018.89	0.9	only tentative assignment
N3H7X2	3380.50	3379.72	*	2048.75	2048.90	0.9	only tentative assignment
N3H7X3	3512.54	ND		2180.79	2180.93	trace	only tentative assignment

Glycopeptide signal intensity:

* -- S/N > 3-5, but <10
** -- S/N > 10
*** -- intense signal, but less intense than "minor"
"significant signal" -- intensity between "minor" and "major"

ND = not detected.

Exhibit G

Glycoforms observed on Asn33 site of HCGβ-only (callus, August sample).

N-Glycan	H-T3 glycopeptide		Comment	Corresponding released free glycan		% of total intensity in MALDI of free glycans	Comment
	m/z theor.	m/z obs.		m/z theor.	m/z obs.		
no glycans	1372.73	1371.82	sequence of deglycosylated peptide was confirmed by PSD	n/a	n/a		
N	1575.81	ND	ND	n/a	n/a		
N2	1778.89	ND	ND	n/a	n/a		
N2H2X	2235.07	ND	ND	903.32	903.24	2.0	
N2H2XF	2381.13	2380.06	significant signal	1049.38	ND	0.0	
N2H3X	2397.13	2396.06	***	1065.38	1065.28	18.2	
N2H3XF	2543.19	2542.10	major	1211.44	1211.32	32.0	
N2H4X	2559.19	2558.12	*	1227.44	1227.30	4.6	overlapping with K-adduct of N2H3XF
N3H3X	2600.21	2599.10	trace	1268.46	1268.35	1.5	
N3H3F	2614.23	ND	ND	1282.48	1282.36	0.6	
N2H3X2F	2675.23	ND	ND	1343.48	1343.37	0.5	only tentative assignment
N2H3XF2	2689.25	ND	ND	1357.50	1357.37	0.6	only tentative assignment
N2H4XF	2705.25	ND	ND	1373.50	1373.37	0.5	
N3H3XF	2746.27	2745.18	***	1414.52	1414.39	8.7	
N3H4X	2762.27	ND	ND	1430.52	1430.37	1.0	overlapping with K-adduct of N3H3XF
N4H3X	2803.16	ND	ND	1471.54	1471.41	0.7	
N3H4XF	2908.33	2907.28	**	1576.58	1576.42	7.6	
N4H3XF	2949.35	2948.27	*	1617.60	1617.45	1.4	
N3H6	2954.35	ND	ND	1622.60	ND	0.0	
N4H4X	2965.35	ND	ND	1633.60	1633.43	0.4	
N3H4X2F	3040.37	ND	ND	1708.62	1708.46	0.5	only tentative assignment
N3H4XF2	3054.39	3053.30	trace	1722.64	1722.46	6.1	
N3H5X2	3056.38	3056.33	trace	1724.63	ND	0.0	only tentative assignment
N3H5XF	3070.39	ND	ND	1738.64	1738.45	1.0	overlapping with K-adduct of N3H4XF2
N4H4XF	3111.41	3111.33	trace	1779.66	1779.48	3.4	
N4H5X	3127.41	ND	ND	1795.66	1795.45	trace	
N3H4X2F2	3186.43	ND	ND	1854.68	1854.49	1.5	only tentative assignment
N2H7XF	3191.43	ND	ND	1859.68	1859.47	trace	
N2H9	3237.46	ND	ND	1905.71	1905.47	0.4	
N4H4XF2	3257.47	ND	ND	1925.72	1925.52	2.4	
N4H5XF	3273.47	ND	ND	1941.72	1941.50	1.9	
N4H4X2F2	3389.51	ND	ND	2057.76	2057.53	0.9	only tentative assignment
N4H5XF2	3419.29	ND	ND	2087.78	2087.54	1.4	
N4H5X2F2	3551.57	ND	ND	2219.82	2219.56	trace	only tentative assignment
N4H5XF3	3565.59	ND	ND	2233.84	2233.57	2.0	
N4H6XF2	3581.59	ND	ND	2249.84	2249.56	trace	overlapping with K-adduct of N4H5XF3
N4H5X2F3	3697.63	ND	ND	2365.88	2365.60	trace	only tentative assignment
N4H5X3F3	3829.67	ND	ND	2497.92	2497.63	trace	only tentative assignment

Glycopeptide signal intensity:

* -- S/N > 3-5, but <10
** -- S/N > 10
*** -- intense signal, but less intense than "minor"
"significant signal" -- intensity between "minor" and "major"

ND = not detected.

Exhibit H

N-glycans released from HCGβ-GalT (callus October sample).

N-Glycan	Mass of released free glycan (sodiated ion)		% of total intensity in MALDI of free glycans	Comment
	m/z theor.	m/z obs.		
NH3X	862.30	862.52	trace	
N2H2X	903.32	903.59	6.0	
N2H3	933.34	933.61	4.4	
NH2X3	964.33	964.59	trace	only tentative assignment (?)
N2HXF(Phos/Sulph)2	1047.27	1047.67	1.9	only tentative assignment (?)
N2H2XF	1049.38	1049.69	1.1	
N2H3X	1065.38	1065.70	14.2	
N2H3F	1079.40	1079.71	1.6	
N2H4	1095.40	1095.71	3.7	
N2H3XF	1211.44	1211.80	23.9	
N2H4X	1227.44	1227.77	8.3	overlapping with K-adduct of N2H3XF
N2H5	1257.46	1257.82	5.6	
N3H3X	1268.46	1268.83	0.5	
N3H4	1298.48	1298.85	0.5	
N2H3X2F	1343.48	1343.87	trace	only tentative assignment (?)
N2H3XF2	1357.50	1357.86	trace	
N2H5X	1389.50	1389.91	1.9	
N2H5F	1403.52	1403.92	1.1	
N3H3XF	1414.52	1414.94	2.9	
N2H6	1419.52	1419.91	0.6	
N3H4X	1430.52	1430.92	1.0	overlapping with K-adduct of N3H3XF
N3H5	1460.54	1460.96	1.1	
N4H3X	1471.54	1471.97	trace	
N2H5XF	1535.56	1536.01	0.3	
N3H4X2	1562.57	1563.03	trace	only tentative assignment (?)
N3H4XF	1576.58	1577.03	1.5	
N3H5X	1592.58	1593.04	0.9	
N4H3XF	1617.60	1618.08	0.5	
N3H6	1622.60	**1623.05**	2.0	
N4H4X	1633.60	1634.08	trace	
N3H4X2F	1708.62	1709.12	trace	only tentative assignment (?)
N3H4XF2	1722.64	1723.14	1.0	
N3H5X2	1724.63	1725.13	0.9	only tentative assignment (?)
N3H5XF	1738.64	1739.14	0.6	overlapping with K-adduct of N3H4XF2
N3H6X	1754.65	1755.13	1.9	
N4H4XF	1779.66	1780.17	0.6	
N3H7	1784.66	1785.16	0.3	
N3H4X2F2	1854.68	1855.20	0.3	only tentative assignment (?)
N3H5X3	1856.67	1857.21	0.3	only tentative assignment (?)
N3H5X2F	1870.68	1871.20	0.3	only tentative assignment (?)
N3H6X2	1886.69	1887.21	1.2	only tentative assignment (?)
N3H6XF	1900.70	1901.23	0.5	
N3H7X	1916.71	1917.22	1.9	
N4H4XF2	1925.72	1926.28	0.3	
N4H5XF	1941.72	1942.25	0.3	
N3H6X3	2018.73	2019.30	0.4	only tentative assignment (?)
N3H7X2	2048.75	2049.30	1.3	only tentative assignment (?)
N4H4X2F2	2057.76	2058.35	trace	only tentative assignment (?)
N3H7X3	2180.79	2181.37	0.3	only tentative assignment (?)
N4H5XF3	2233.84	2234.46	0.3	only tentative assignment (?)
N4H6XF2	2249.84	2250.43	trace	overlapping with K-adduct of N4H5XF3
N8H4	2313.88	2313.42	trace	only tentative assignment (?)
N7H3X3	2344.87	2344.48	trace	only tentative assignment (?)
N2H10F3	2505.94	2505.57	trace	only tentative assignment (?)
N2H11F3	2668.00	2667.65	trace	only tentative assignment (?)

ND = not detected

Exhibit I

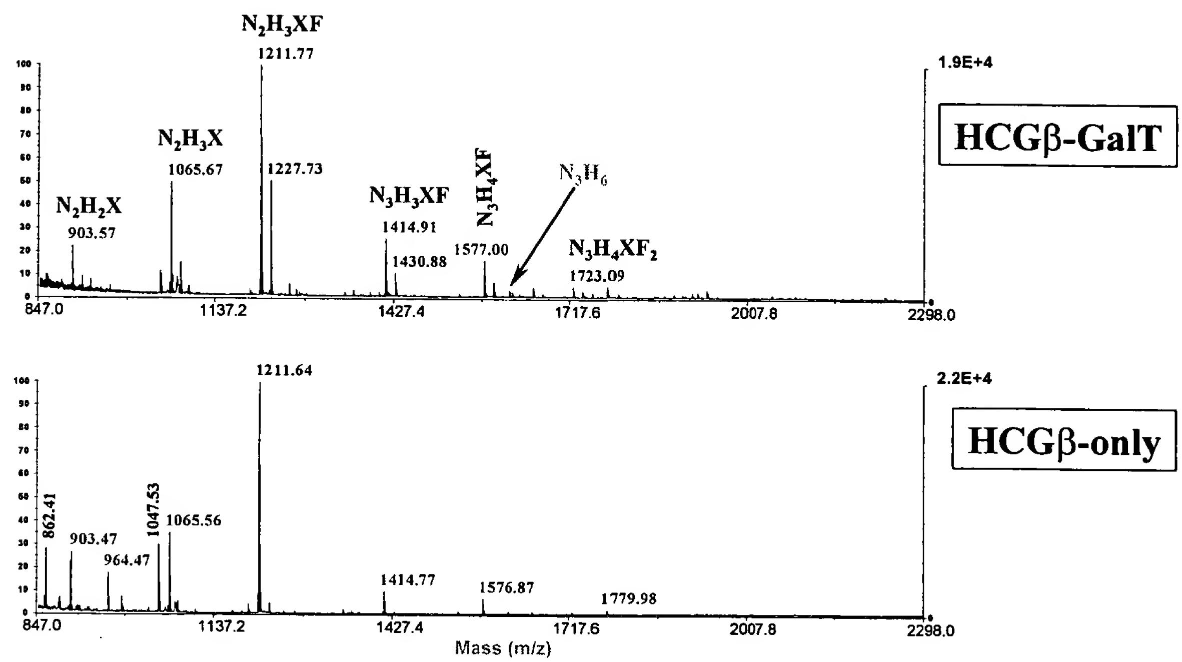
N-glycans released from HCGβ-only (callus, October sample).

N-Glycan	Mass of released free glycan (sodiated ion)		% of total intensity in MALDI of free glycans	Comment
	m/z theor.	m/z obs.		
N2H2X	903.32	903.45	3.4	
N2H3	933.34	933.46	trace	
N2HXF(Phos/Sulph)2	1047.27	1047.52	2.3	only tentative assignment (?)
N2H2XF	1049.38	1049.54	1.8	
N2H3X	1065.38	1065.54	17.7	
N2H3F	1079.40	1079.54	1.3	
N2H3XF	1211.44	1211.62	37.0	
N2H4X	1227.44	1227.59	12.0	overlapping with K-adduct of N2H3XF
N3H3X	1268.46	1268.64	0.9	
N2H3X2F	1343.48	1343.68	trace	only tentative assignment (?)
N2H3XF2	1357.50	1357.71	trace	
N3H3XF	1414.52	1414.71	5.5	
N3H4X	1430.52	1430.70	1.8	overlapping with K-adduct of N3H3XF
N4H3X	1471.54	1471.74	trace	
N3H4XF	1576.58	1576.78	3.8	
N3H5X	1592.58	1592.77	0.9	
N4H3XF	1617.60	1617.81	0.9	
N3H6	1622.60	**ND**	0.0	
N4H4X	1633.60	1633.81	0.4	
N3H4XF2	1722.64	1722.86	2.5	
N3H5XF	1738.64	1738.85	1.0	overlapping with K-adduct of N3H4XF2
N3H6X	1754.65	1754.88	trace	
N4H4XF	1779.66	1779.87	1.4	
N3H4X2F2	1854.68	1854.91	0.6	only tentative assignment (?)
N3H5X2F	1870.68	1870.88	trace	only tentative assignment (?)
N4H4XF2	1925.72	1925.94	0.9	
N4H5XF	1941.72	1941.95	0.9	
N4H4X2F2	2057.76	2057.99	0.3	only tentative assignment (?)
N4H5XF2	2087.78	2088.01	0.4	
N4H5XF3	2233.84	2234.06	0.9	only tentative assignment (?)
N4H6XF2	2249.84	2250.08	trace	overlapping with K-adduct of N4H5XF3

ND = not detected

Exhibit J

Exhibit K



Plant, October samples. A comparison of representative MALDI MS profiles of N-glycans released form plant-expressed HCGβ-GalT (upper panel) and HCGβ-only (lower panel) samples. Assignments for some m/z peaks are shown in the figure. N3H6 glycan (m/z ~ 1623) appears with minor intensity (~0.5% of the sum of peak intensities) in HCGβ-GalT sample, whereas it is absent in HCGβ-only sample. M/z 1623 glycan peak is not the only difference between the HCGβ-GalT and HCGβ-only glycan profiles, but m/z 1623 glycan is the only one that looses galactose residue upon β(1-3,4)-galactosidase treatment.

N-glycans released from HCGβ-GalT (plant, October sample).

N-Glycan	Mass of released free glycan (sodiated ion)		% of total intensity in MALDI of free glycans	Comment
	m/z theor.	m/z obs.		
N2H2X	903.32	903.57	5.6	
N2H3	933.34	933.57	1.5	
NH2X3	964.33	964.57	trace	only tentative assignment (?)
N2HXF(Phos/Sulph)2	1047.27	1047.65	2.9	only tentative assignment (?)
N2H2XF	1049.38	1049.65	2.5	
N2H3X	1065.38	1065.67	13.8	
N2H3F	1079.40	1079.66	trace	
N2H4	1095.40	1095.67	1.1	
N2H3XF	1211.44	1211.77	28.3	
N2H4X	1227.44	1227.73	13.9	overlapping with K-adduct of N2H3XF
N2H5	1257.46	1257.79	1.5	
N3H3X	1268.46	1268.80	0.8	
N2H4XF	1373.50	1373.86	trace	
N2H5X	1389.50	1389.87	0.5	
N2H5F	1403.52	1403.89	0.5	
N3H3XF	1414.52	1414.91	7.1	
N3H4X	1430.52	1430.88	2.8	overlapping with K-adduct of N3H3XF
N3H5	1460.54	1460.92	trace	
N4H3X	1471.54	1471.94	trace	
N2H5XF	1535.56	1535.95	0.4	
N3H4XF	1576.58	1577.00	4.4	
N3H5X	1592.58	1592.98	1.7	
N4H3XF	1617.60	1618.03	0.8	
N3H6	1622.60	1623.02	0.5	
N4H4X	1633.60	1634.02	0.4	
N3H4XF2	1722.64	1723.09	1.3	
N3H5XF	1738.64	1739.09	0.8	
N4H4XF	1779.66	1780.13	1.3	
N4H5X	1795.66	1796.11	0.5	
N3H5X3	1856.67	1855.18	trace	only tentative assignment (?)
N3H5X2F	1870.68	1871.15	trace	
N3H6X2	1886.69	1887.16	0.4	only tentative assignment (?)
N3H6XF	1900.70	1901.19	trace	
N2H9	1905.71	1906.19	trace	
N3H7X	1916.71	1917.19	0.6	
N4H4XF2	1925.72	1926.23	0.6	
N4H5XF	1941.72	1942.22	0.8	
N3H6X3	2018.73	2019.25	trace	only tentative assignment (?)
N3H7X2	2048.75	2049.26	0.5	only tentative assignment (?)
H3H7X3	2180.79	2181.38	trace	only tentative assignment (?)
N4H5XF3	2233.84	2234.41	0.4	only tentative assignment (?)
N4H6XF2	2249.84	2250.40	trace	overlapping with K-adduct of N4H5XF3

ND = not detected

Exhibit L

N-glycans released from HCGβ-only (plant, October sample)

N-Glycan	Mass of released free glycan (sodiated ion)		% of total intensity in MALDI of free glycans	Comment
	m/z theor.	m/z obs.		
NH3X	862.30	862.41	9.4	
N2H2X	903.32	903.47	8.8	
N2H3	933.34	933.48	0.7	
NH2X3	964.33	964.47	5.9	only tentative assignment (?)
N2HXF(Phos/Sulph)2	1047.27	1047.53	10.3	only tentative assignment (?)
N2H2XF	1049.38	1049.54	4.1	
N2H3X	1065.38	1065.56	12.1	
N2H3F	1079.40	1079.56	1.8	
N2H3XF	1211.44	1211.64	35.1	
N2H4X	1227.44	1227.61	trace	overlapping with K-adduct of N2H3XF
N3H3X	1268.46	1268.69	trace	
N2H4XF	1373.50	1373.74	trace	
N3H3XF	1414.52	1414.77	3.4	
N3H4X	1430.52	1430.79	trace	overlapping with K-adduct of N3H3XF
N2H5XF	1535.56	1535.83	0.5	
N3H4XF	1576.58	1576.87	2.4	
N4H3XF	1617.60	1617.92	0.4	
N3H6	1622.60	ND	0.0	
N3H4XF2	1722.64	1722.95	0.4	
N4H4XF	1779.66	1779.96	0.6	
N4H4XF2	1925.72	1926.09	trace	
N4H5XF	1941.72	1942.08	0.3	
N4H5XF2	2087.78	2088.18	trace	

ND = not detected

Exhibit M

EXHIBIT N

Summary on N-Glycan Analysis for all HCG□ Samples Studied

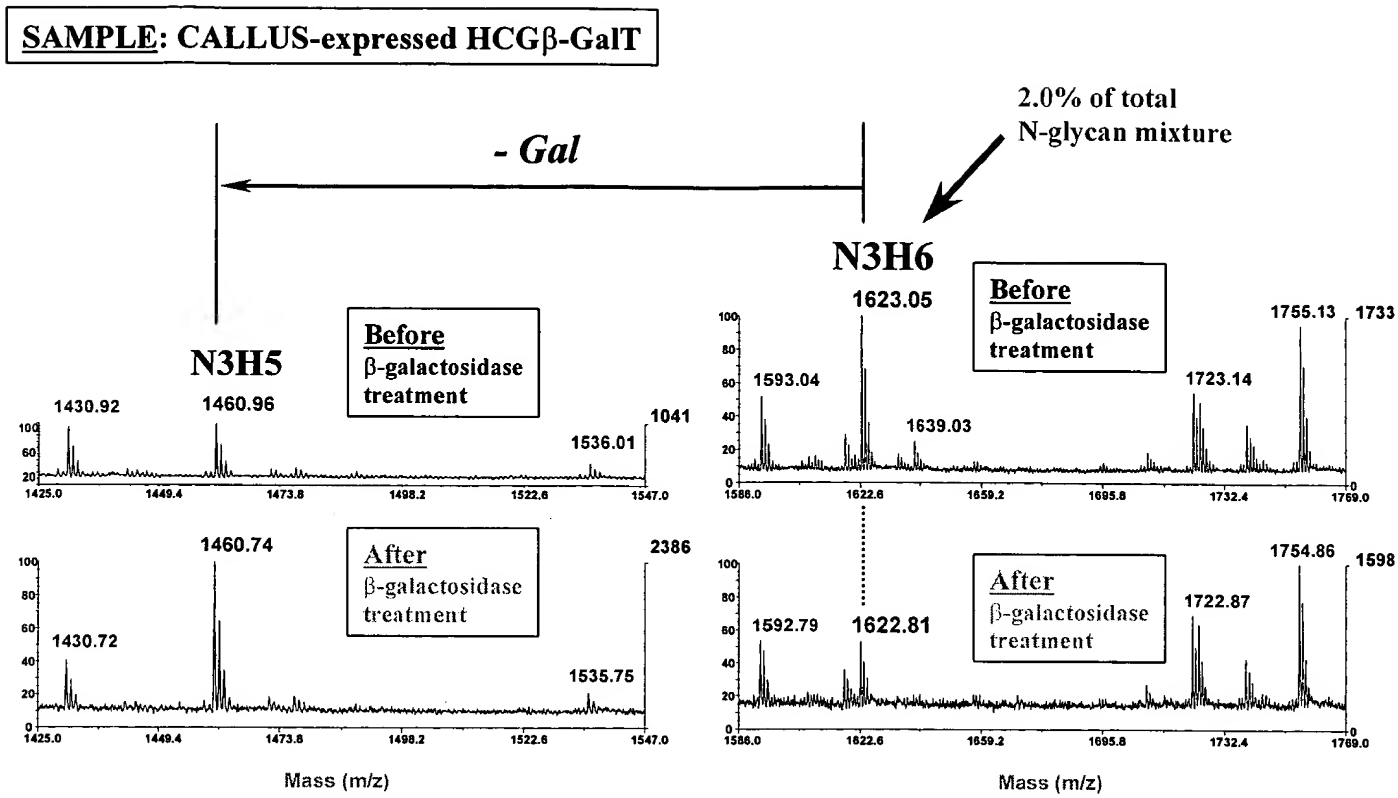
N-Glycan	m/z theor. (free glycan)	% abundance from MALDI of free glycans					
		HCGβ-only, Callus, August	HCGβ-only, Callus, October	HCGβ-only, Plant, October	HCGβ-GalT, Callus, August	HCGβ-GalT, Callus, October	HCGβ-GalT, Plant, October
NH3X	862.30	ND	ND	9.4	ND	trace	ND
N2H2X	903.32	2.0	3.4	8.8	6.1	6.0	5.6
N2H3	933.34	ND	trace	0.7	trace	4.4	1.5
NH2X3	964.33	ND	ND	5.9	ND	trace	trace
N2HXF(Phos/Sulph)2	1047.27	ND	2.3	10.3	ND	1.9	2.9
N2H2XF	1049.38	ND	1.8	4.1	ND	1.1	2.5
N2H3X	1065.38	18.2	17.7	12.1	22.7	14.2	13.8
N2H3F	1079.40	ND	1.3	1.8	ND	1.6	trace
N2H4	1095.40	ND	ND	ND	2.7	3.7	1.1
N2H3XF	1211.44	32.0	37.0	35.1	24.7	23.9	28.3
N2H4X	1227.44	4.6	12.0	trace	8.9	8.3	13.9
N2H5	1257.46	ND	ND	ND	6.2	5.6	1.5
N3H3X	1268.46	1.5	0.9	trace	ND	0.5	0.8
N3H3F	1282.48	0.6	ND	ND	ND	ND	ND
N3H4	1298.48	ND	ND	ND	ND	0.5	ND
N2H3X2F	1343.48	0.5	trace	ND	ND	trace	ND
N2H3XF2	1357.50	0.6	trace	ND	ND	trace	ND
N2H4XF	1373.50	0.5	ND	trace	ND	ND	trace
N2H5X	1389.50	ND	ND	ND	3.3	1.9	0.5
N2H5F	1403.52	ND	ND	ND	trace	1.1	0.5
N3H3XF	1414.52	8.7	5.5	3.4	7.4	2.9	7.1
N2H6	1419.52	ND	ND	ND	ND	0.6	ND
N3H4X	1430.52	1.0	1.8	trace	1.0	1.0	2.8
N3H5	1460.54	ND	ND	ND	1.0	1.1	trace
N4H3X	1471.54	0.7	trace	ND	ND	trace	trace
N2H5XF	1535.56	ND	ND	0.5	ND	0.3	0.4
N3H4X2	1562.57	ND	ND	ND	1.6	trace	ND
N3H4XF	1576.58	7.6	3.8	2.4	2.9	1.5	4.4
N3H5X	1592.58	ND	0.9	ND	ND	0.9	1.7
N4H3XF	1617.60	1.4	0.9	0.4	ND	0.5	0.8
N3H6	1622.60	ND	ND	ND	1.1	2.0	0.5
N4H4X	1633.60	0.4	0.4	ND	ND	trace	0.4
N3H4X2F	1708.62	0.5	ND	ND	1.0	trace	ND
N3H4XF2	1722.64	6.1	2.5	0.4	ND	1.0	1.3
N3H5X2	1724.63	ND	ND	ND	2.3	0.9	ND
N3H5XF	1738.64	1.0	1.0	ND	ND	0.6	0.8

Continued

EXHIBIT N (continued)

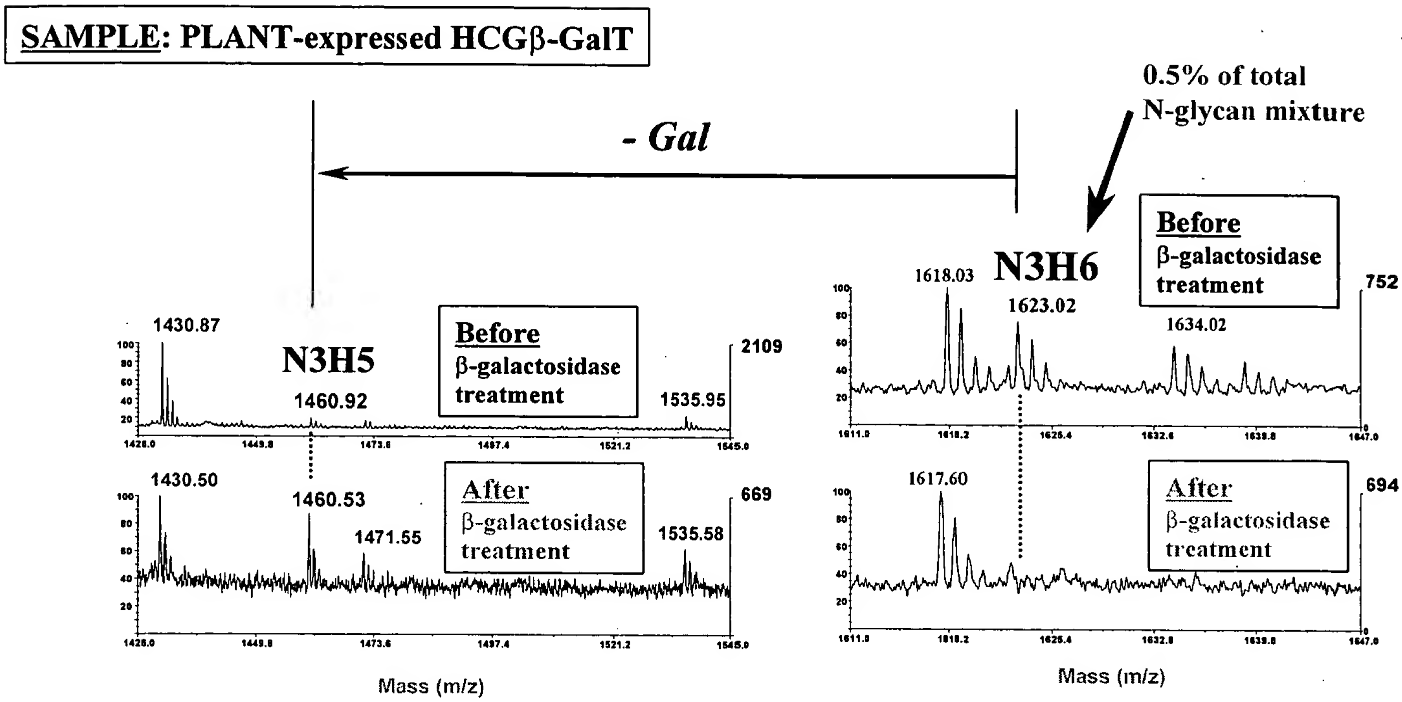
N-Glycan	m/z theor. (free glycan)	% abundance from MALDI of free glycans					
		HCGβ-only, Callus, August	HCGβ-only, Callus, October	HCGβ-only, Plant, October	HCGβ-GalT, Callus, August	HCGβ-GalT, Callus, October	HCGβ-GalT, Plant, October
N3H6X	1754.65	ND	trace	ND	2.3	1.9	ND
N4H4XF	1779.66	3.4	1.4	0.6	ND	0.6	1.3
N3H7	1784.66	ND	ND	ND	ND	0.3	ND
N4H5X	1795.66	trace	ND	ND	ND	ND	0.5
N3H4X2F2	1854.68	1.5	0.6	ND	ND	0.3	trace
N3H5X3	1856.67	ND	ND	ND	1.2	0.3	ND
N2H7XF	1859.68	trace	ND	ND	ND	ND	ND
N3H5X2F	1870.68	ND	trace	ND	ND	0.3	trace
N3H6X2	1886.69	ND	ND	ND	2.1	1.2	0.4
N3H6XF	1900.70	ND	ND	ND	ND	0.5	trace
N2H9	1905.71	0.4	ND	ND	ND	ND	trace
N3H7X	1916.71	ND	ND	ND	0.8	1.9	0.6
N4H4XF2	1925.72	2.4	0.9	trace	ND	0.3	0.6
N4H5XF	1941.72	1.9	0.9	0.3	ND	0.3	0.8
N3H6X3	2018.73	ND	ND	ND	0.9	0.4	trace
N3H7X2	2048.75	ND	ND	ND	0.9	1.3	0.5
N4H4X2F2	2057.76	0.9	0.3	ND	ND	trace	ND
N4H5XF2	2087.78	1.4	0.4	trace	ND	ND	ND
N3H7X3	2180.79	ND	ND	ND	trace	0.3	trace
N4H5X2F2	2219.82	trace	ND	ND	ND	ND	ND
N4H5XF3	2233.84	2.0	0.9	ND	ND	0.3	0.4
N4H6XF2	2249.84	trace	trace	ND	ND	trace	trace
N8H4	2313.88	ND	ND	ND	ND	trace	ND
N7H3X3	2344.87	ND	ND	ND	ND	trace	ND
N4H5X2F3	2365.88	trace	ND	ND	ND	ND	ND
N4H5X3F3	2497.92	trace	ND	ND	ND	ND	ND
N2H10F3	2505.94	ND	ND	ND	ND	trace	ND
N2H11F3	2668.00	ND	ND	ND	ND	trace	ND

ND = not detected
WT = wild-type (no GalT-I gene expressed)
GalT = co-expressed with GalT-I gene



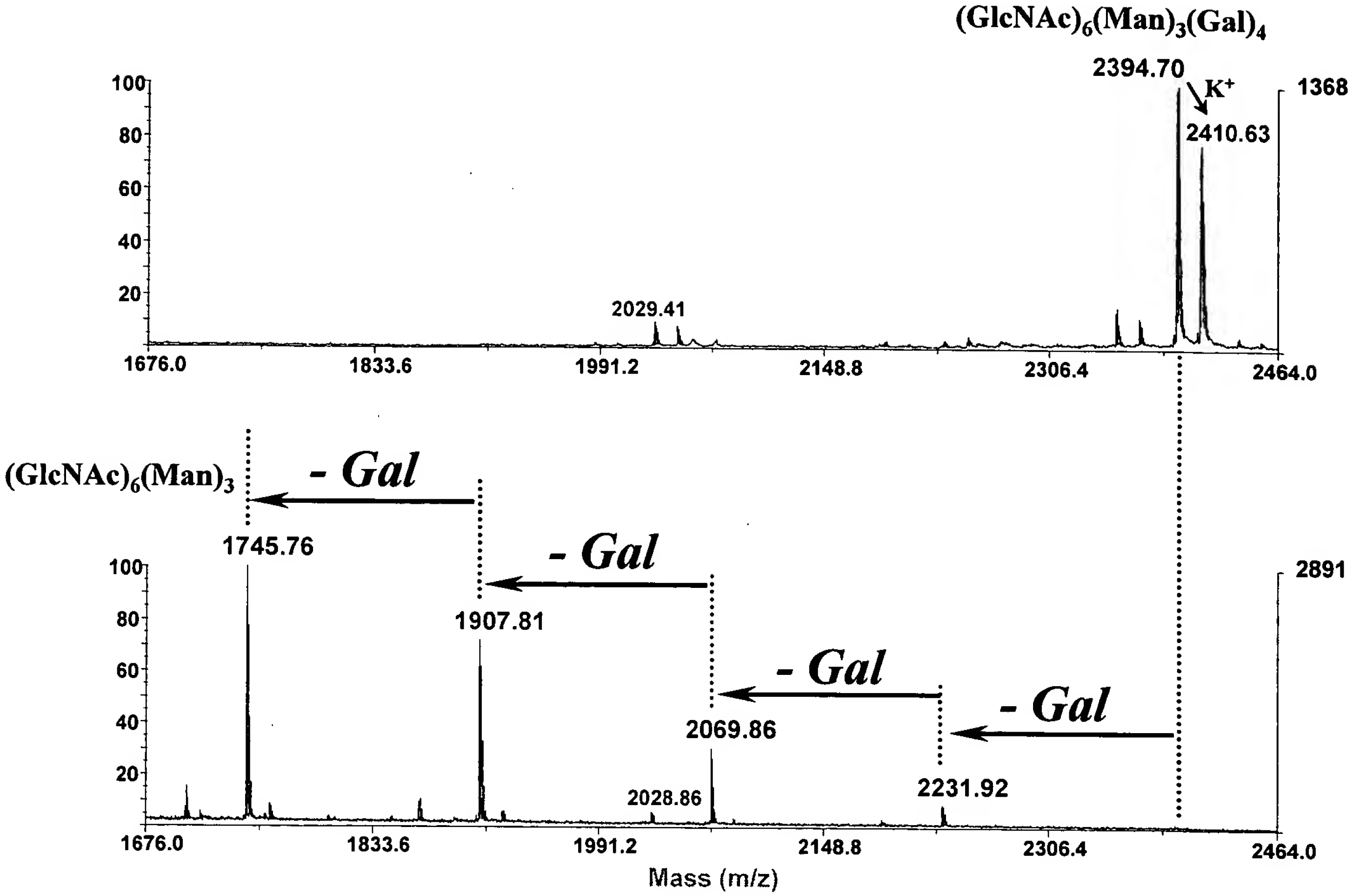
Removal of β(1,4)-Gal from N3H6 glycan of callus-expressed HCGβ-GalT sample. Upper panels: before treatment with β(1-3,4)-galactosidase. Lower panels: after treatment with β(1-3,4)-galactosidase. Upon β-galactosidase treatment, relative intensity of m/z 1623 (N3H6 glycan) decreases, while relative intensity of m/z 1460 (N3H5 glycan) increases. This suggests that removal of one Gal residue takes place.

Exhibit O



Removal of β(1,4)-Gal from N3H6 glycan of plant-expressed HCGβ-GalT sample. Upper panels: before treatment with β(1-3,4)-galactosidase. Lower panels: after treatment with β(1-3,4)-galactosidase. Upon β-galactosidase treatment, relative intensity of m/z 1623 (N3H6 glycan) decreases, while relative intensity of m/z 1460 (N3H5 glycan) increases. This suggests that removal of one Gal residue takes place.

Exhibit P



A control experiment was performed to test efficiency of β-galactosidase reaction. NA4 N-glycan standard, containing four terminal β(1,4)-galactose residues (see MALDI spectrum in upper panel), was treated with β(1-3,4)-galactosidase and the reaction products were examined by MALDI MS (lower panel). Complete conversion of the tetra-galactosylated glycan to its degalactosylated products was observed. The most abundant degalactosylated species was glycan with all four Gal residues removed (m/z 1745.76).

Exhibit Q